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Evaluation of the Mythic 18,

haematology analyser for its use in dogs, cats and horses

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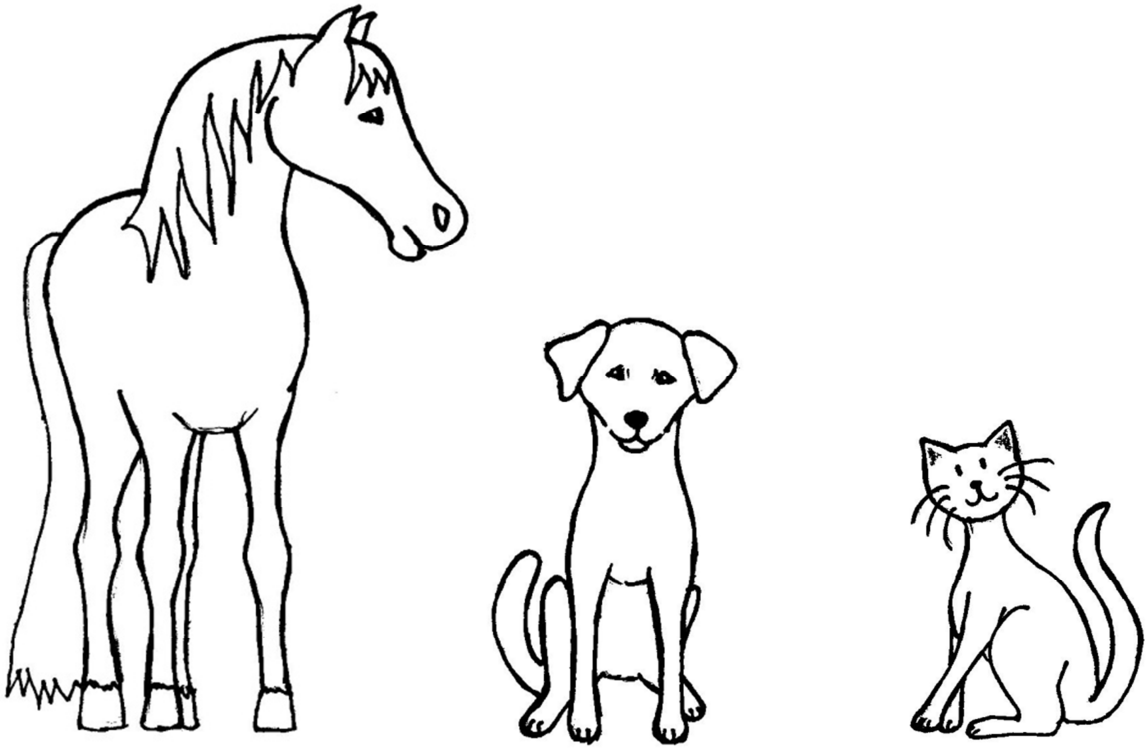
In Liebe und Dankbarkeit meinen Eltern gewidmet,

ohne die ich nicht bist hier gekommen wäre.



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1. Abstract

The Mythic 18 is a fully automated haematology bench-top analyser using impedance technology for a complete blood count (CBC) and a 3-part white blood cell (WBC) differential. The purpose of this study was to evaluate the Mythic for accuracy, precision, linearity, carry-over, stability and usability under practice conditions. EDTA-blood samples from 122 dogs, 140 cats and 123 horses were analysed with the Mythic and reference methods (Sysmex XT-2000iV, manual haematocrit and microscopic WBC differentiation). Red blood cell parameters showed excellent correlation and small biases. Total WBC count correlated excellently in canine and equine and very well in feline samples. In 23 feline specimens with platelet aggregates, the Mythic overestimated WBC counts. In all three species, absolute granulocyte counts correlated excellently. Equine lymphocyte counts showed good correlation whereas canine and feline lymphocyte counts correlated poorly. Feline platelets showed good correlation with a negative bias. The instrument showed good to excellent precision and performed excellently for the CBC count parameters in all investigated species. The whole 3-part differential was found to be accurate in horses. In dogs and cats absolute granulocyte counts were reliable. As with all impedance based haematological instruments, evaluation of a blood smear is absolutely indicated to check for the presence of platelet aggregates, to verify WBC differentiation and to identify possible pathologies.

2. Introduction

Haematological results provide important information on the patient's state of health, disease history and response to treatment (Wenger-Riggenbach et al., 2006). The invention of the Coulter cell counter and cell volume analyser in 1956 highly reduced time-consuming manual work by automating the counting and sizing of cells (Knoll, 2000). Since then, several affordable, automated bench-top haematology analysers have been developed for in-clinic use (Becker et al., 2008). Most of these analysers are primarily designed for human blood. When analysing nonhuman haematology specimens, it is essential that the selected instrument be designed and validated for multispecies analysis (Weiser, 1987a).

The Mythic 18 (Orpée SA, Geneva, Switzerland) is an impedance-based haematology instrument originally designed for human application. To make the instrument suitable for veterinary application, settings for feline, canine and equine blood samples have been developed in the Clinical laboratory, Vetsuisse-Faculty University of Zurich. This evaluation was conducted to assure the quality of the newly designed animal settings. Information on imprecision and inaccuracy of a haematological instrument are extremely valuable for the users. Each type of instrument should therefore be validated for each species before using results for clinical purpose.

The objective of the present study was to validate the Mythic 18 for use with blood samples from healthy and diseased cats, dogs and horses. To this end, accuracy, precision, linearity, carry over and sample stability were determined. Biases were judged with respect to their clinical relevance.

3. Material and Methods

3.1 Blood samples

Fresh EDTA-K_3 blood samples from 122 dogs, 140 cats and 123 horses from the Small Animal Clinic and the Clinic for horses, at the Vetsuisse-Faculty, University of Zurich were analysed on the Sysmex XT-2000iV and reference methods, and usually with a time delay of 1.5 hours (until the routine work was finished) on the Mythic 18. All samples were collected by venipuncture regardless of sex, age or breed and sent to the clinical laboratory in the framework of routine work to check the health status. Sample collection took place between May and December 2009. Complete sample analysis was performed within 6 hours after collection, most of them within 4 hours. The aforementioned blood samples were used to assess accuracy and precision. To determine the range of linear measurement, 2 blood samples from cats, 2 from dogs and 1 equine blood sample were used. Additionally, platelet enriched plasma from a horse was used to assess linearity of the platelet count. Carry-over of blood from one sample to the following sample, meaning the effectiveness of cleaning of the instrument, was assessed for each species using 2 EDTA-blood samples. To determine the effect of aging of samples, blood samples from 6 dogs and cats and 8 horses were used.

3.2 Instruments and methods used

3.2.1 Mythic 18

Mythic 18 (Orpée SA, Geneva, Switzerland) is a fully automated in house haematology analyser performing haematological analyses on EDTA-anticoagulated blood.

***Figure 1*: Mythic 18**

The instrument is used widely in human medicine, with more than 4.000 instruments worldwide. Recently, the software has been adapted for veterinary use. Species profiles for cats, dogs and horses were installed in the author's laboratory. In total, 19 species profiles can be created.

For counting the cellular blood components, the Mythic 18 uses the impedance technique only. A cyanide-free spectrophotometry method is used to measure haemoglobin by formation of oxyhaemoglobin at 555 nm. Haematocrit is measured by volume integration. The sample volume is 10 µl. The instrument can determine 16 parameters in the normal mode and 18 in the research mode: white blood cells (WBC) with absolute number and percentage of lymphocytes (LYM), monocytes (MONO) and granulocytes (GRAN), number of red blood cells (RBC), haemoglobin concentration (HGB), haematocrit value (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV) and for research plateletcrit (PCT) and platelet distribution width (PDW). For platelet counting a floating threshold is used, whereas for RBC and WBC counts the thresholds are predefined. Results are provided within 1 minute on the LCD display, printed out on the printer and stored in the resident memory or in an USB key. Results were presented with flags; optionally reference ranges can be reported. Additionally, the Mythic 18 shows histograms for WBC, RBC and PLT. Prior to analysis, patient's data can be entered manually or with a barcode reader. The instrument also displays message codes and histogram flags. However, they have not been adapted yet to feline, canine and equine blood samples. Therefore conclusions about the usefulness of these message codes and flags cannot be drawn at this time.

Mythic 18 provides a 3-part WBC differential in samples with WBC counts in the range between $0.9 \times 10^3/\mu l$ and $150 \times 10^3/\mu l$. Quality control samples are supplied as blood samples with 3 levels of RBC, WBC and PLT levels (Myt-3D, lots B059, B089, B119, Orphée S.A., Geneva, Switzerland). Results of each lot can be viewed on the display of the instrument in

tables and Levey-Jennings graphs. The instrument uses three reagents: a diluent, a lysis reagent and a cleaning solution (Mythic 18 Vet M-Pack, Orphée S.A., Geneva, Switzerland).

3.2.2 Sysmex XT-2000iV

The Sysmex XT-2000iV (Sysmex Corporation, Kobe, Japan), equipped with the software version 10b, was used as the reference instrument for total WBC count, WBC-differentiation, RBC count, RBC-indices, HGB, RDW, PLT count and MPV. It is a fully automated haematology analyser for animal blood providing 30 parameters. The impedance method with hydrodynamic focusing is used for RBC (RBC-I), HCT and PLT (PLT-I). With these results MCV, MCH, MCHC, RDW, MPV and PDW are calculated. A flow cytometry device based on a sheath-flow and a semiconductor laser is used as an optical method for platelets (PLT-O) in cats, WBC counts and differentiation. HGB is measured spectrophotometrially with a cyanide-free (sodiumlaurylsulfat) method.

3.2.3 Manual methods

Manual HCT measurement was done with microhaematocrit capillary tubes centrifuged at 13.000 g for 5 min in a microhaematocrit centrifuge (Knoll and Rowell, 1996).

Blood smears were stained using an automated staining instrument (HemaTek, Siemens). Microscopic differentiation of two modified Wright-stained blood smears, 100 WBC each, was done by 2 technicians with 10 years experience in veterinary haematology each. These results were used to calculate the absolute number of LYM, MONO and GRAN counts by multiplying the percentage from the 200-cell count of each cell type with the total WBC count from the Sysmex XT-2000iV.

3.2.4 Accuracy

Analytical accuracy is defined by the International Council for Standardization in Haematology (ICSH) as a measure of agreement between the measured value of an analyte and its “true” value (ICSH, 1994). To determine accuracy, agreement between the results of the evaluated instrument and the results of a reference instrument were compared. In this study, accuracy was determined by comparing the results of the Mythic 18 with those of the reference instrument, the manual HCT and the microscopic differentiation. Sysmex XT-2000iV is widely used and accepted in veterinary clinical laboratories and validation studies were conducted on the Sysmex XT-2000iV for cats (Weissenbacher et al., (2010)) and cats, dogs and horses (Lilliehöök and Tvedten, 2009a, b). For comparing results of granulocytes of the Mythic 18, results of the neutrophils, eosinophils and basophils of the reference methods were added.

3.2.5 Precision

Within-series precision of the instrument was determined for each of the investigated species for low, normal and high WBC-values based on multiple analyses (more than 12 consecutive times). During the analysis the sample was gently mixed. Afterwards mean, standard deviation and coefficient of variation as a measurement of the random error were calculated for all parameters.

Precision from day to day was measured using commercially available (Myt-3D, Orphée S.A., Geneva, Switzerland) quality control blood of low, intermediate and high levels, which were analysed once daily prior to analysing patient's samples over a 20-day period.

3.2.6 Linearity

The linearity of the measurement range was assessed in all four species to determine the analytical range. Mythic 18 has a reportable range for WBC (-150x10^3/µl), for RBC (-15x10^6/µl), for HCT (-72%) and for PLT (-4.000x10^3/µl). The linearity of the measurement range was determined for WBC, RBC, HCT, HGB and PLT by analysing a series dilution of K_3-EDTA anticoagulated blood in triplicate. For cat and dog two blood samples were used, one with high WBC counts to determine WBC linearity (5ml) and one (cat 12 ml, dog 10 ml) for the remaining parameters. One equine sample (20 ml ETDA-blood) was used for RBC, HGB and HCT, additionally platelet enriched plasma of a horse was used to determine PLT linearity. The blood samples were centrifuged at 390 g for ten minutes (Rotina 35 R, Hettrich AG) to receive results below and above the reference range. Then the plasma was removed from the blood cells. Afterwards concentrated blood cells were diluted with 0.9% saline solution in steps of 10%, to achieve a dilution series from 0% up to 100% blood cell concentrate.

3.2.7 Carry-over

Carry-over was studied to assess if transfer of blood from one sample will cause a falsely higher result in the following sample. For each species, 2 patient samples, one with high WBC counts, were analysed 2 times followed by 3 replicates of diluents (Laboratory Equipment and Methods Advisory Group, 1969).

3.2.8 Cell aging

Cell aging studies were performed with blood samples from 6 cats, 6 dogs and 8 samples from horses. They were analysed at time point 1, 2, 4, 6, 8, 24, 32 and 48 hours after collection to calculate stability. The blood was stored at room temperature during the whole experiment. For each parameter, the difference in mean of results between each analysis and time point 1 hour was calculated. In cats only RBC parameter were investigated.

3.2.9 Statistical analysis

All data were entered manually in a Microsoft Excel spreadsheet (Microsoft Excel 2007, Microsoft Corp., Redmond, WA, USA). The Microsoft Excel Add in Analyse-it (Analyse-it Software Ltd., Leeds, UK) was used for statistical analyses. For each parameter and each investigated species, Pearson's coefficient of correlation (*r*), linear regression analysis according Passing and Bablok providing intercept and slope with the 95% confidence interval and Bland Altman Difference Plot with biases and 95% limits of agreement were calculated. Pearson´s coefficient of correlation measures the amount of linear association between the results of two methods on the x and y axis. Coefficient of correlation was considered excellent if $r \geq 0.95$, very good if $r = 0.90\text{-}0.94$, good if $r = 0.80\text{-}0.89$, fair if $r = 0.59\text{-}0.79$ and poor if $r < 0.59$ (Welles et al., 2009). In the Passing-Bablok regression analysis, results of the reference method and the tested instrument are plotted on the x and y axis and a best fit

regression line is calculated and compared to the line of identity. This statistical analysis allows imprecision in both compared methods and is robust against outliers (Bablok and Passing, 1985). The calculated slope shows the proportional systematic error while the intercept shows the constant systematic error (Tvedten and Korcal, 1996). In the Bland Altman Difference Plot, the difference between the results of the two methods is plotted against the average of the two measurements. The presented bias reflects the systematic error; it is calculated by the reference method value minus the Mythic 18 result (Altman and Bland, 1983).

For precision analysis, standard deviation (SD) and Coefficient of variation (CV) were calculated for each level, parameter and species. CV was computed with formula:

$$Coefficient\ of\ variation = \frac{standard\ deviation \times 100}{mean}$$

The degree of linearity was determined with Analyse-it according to Emancipator-Kroll (Kroll and Emancipator, 1993).

For WBC, RBC, HGB and PLT the percentage of carry-over was calculated by the formula:

$$\%\ carry - over = \frac{(Result\ empty\ cycle\ 1) - (Result\ empty\ cycle\ 3)}{(Sample\ 2) - (Result\ empty\ cycle\ 3)} \times 100$$

The stability of the blood samples was reviewed for statistical significant changes using the Friedman-Test and the Dunn`s Multiple Comparison post test (GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). Statistical significance was tested for the result of the first hour compared with the results of the following time points. Statistical significance was defined as *p* value <0.05.

3.3 Clinical relevance

For each sample, the data from the Mythic 18 and the reference methods were compared with established haematology reference values, used in the Clinical Laboratory of the Vetsuisse-Faculty University of Zurich (Table 1). The results were judged to be below or above the reference range and the resulting interpretations from the Mythic 18 and the reference methods were compiled and compared to each other.

Table 1: ***Reference values of haematological parameters for cats, dogs and horses used in this study***

Parameter	Cat	Dog	Horse
WBC (x10³/µl)	4.6 - 12.8	4.7 - 11.3	4.7 - 8.2
LYM (/µl)	1050 - 6000	1154 - 3399	1020 - 3472
MONO (/µl)	45 - 678	198 - 917	0 - 184
BANDS (/µl)	0 - 123	0 - 84	0 - 75
NEUTOPHILS (/µl)	2315 - 10.011	2496 - 7437	3021 - 5775
EOSINOPHILS (/µl)	100 - 600	119 - 1287	0 - 216
BASOPHILS (/µl)	0 - 143	0 - 82	0 - 66
RBC (x10⁶/µl)	7 - 10.7	6.1 - 8.1	6.2 - 9
HGB (g/dl)	11.3 - 15.5	14.4 - 19.1	10.8 - 14.9
HCT (%)	33 - 45	42 - 55	30 - 42
MCH (pg)	14 - 17	23 - 26	15 - 18
MCV (fl)	41 - 49	64 - 73	41 - 50
MCHC (g/dl)	33 - 36	34 - 36	35 - 37
PLT (x10³/µl)	180 - 680	130 - 394	119 - 250

4. Results

4.1 Accuracy

Pearson`s coefficient of correlation, intercept and slope with 95% confidence intervals (CI) calculated by Passing-Bablok regression analysis, and biases with their 95% limits of agreement calculated by Bland-Altman Difference Plot are presented in Table 2 and 3. Table 2 shows results for WBC, RBC and PLT, Table 3 presents results of the WBC differentiation compared with results from the Sysmex XT-2000iV and results of the manual WBC differentiation.

Table 2: ***Accuracy results from the Mythic 18, compared with the results of the reference methods***

Parameter	Species	Coefficient of correlation	Intercept (95% CI)	Slope (95% CI)	Bias (95% Limits of agreement)	Number of samples
WBC	Cat	0.94	0.26 (-0.14 to 0.61)	0.91 (0.88 to 0.95)	-0.072 (-6.959 to 6.815)	129
	Dog	0.99	0.98 (0.68 to 1.36)	0.95 (0.92 to 0.98)	0.229 (-3.651 to 4.110)	122
	Horse	0.98	0.38 (0.17 to 0.56)	0.94 (0.92 to 0.97)	-0.126 (-1.496 to 1.243)	123
RBC	Cat	0.99	0.51 (0.37 to 0.66)	0.95 (0.93 to 0.97)	0.090 (-0.400 to 0.581)	138
	Dog	0.99	0.26 (0.15 to 0.40)	1.00 (0.98 to 1.02)	0.241 (-0.096 to 0.578)	122
	Horse	0.98	0.38 (0.21 to 0.57)	0.93 (0.91 to 0.96)	-0.140 (-0.693 to 0.414)	123
HGB	Cat	0.99	0.38 (0.21 to 0.54)	0.92 (0.90 to 0.93)	-0.511 (-1.256 to 0.234)	138
	Dog	1.00	1.02 (0.81 to 1.26)	0.93 (0.92 to 0.95)	0.10 (-0.57 to 0.76)	122
	Horse	0.98	0.48 (0.08 to 0.76)	0.94 (0.92 to 0.98)	-0.25 (-1.09 to 0.58)	123
HCT	Cat	0.99	2.05 (1.23 to 2.74)	0.94 (0.92 to 0.97)	0.16 (-2.15 to 2.48)	135
	Dog	0.99	1.20 (0.05 to 2.13)	0.95 (0.93 to 0.98)	-0.79 (-3.42 to 1.83)	121
	Horse	0.99	1.36 (0.43 to 2.13)	0.96 (0.93 to 0.98)	-0.17 (-1.95 to 1.61)	123
MCV	Cat	0.95	4.65 (2.50 to 7.12)	0.91 (0.85 to 0.96)	0.86 (-2.62 to 4.33)	138
	Dog	0.96	8.01 (4.54 to 11.37)	0.83 (0.78 to 0.88)	-3.16 (-6.03 to -0.28)	122
	Horse	0.94	3.62 (1.08 to 5.82)	0.90 (0.85 to 0.95)	-1.02 (-3.68 to 1.63)	123
MCH	Cat	0.97	-0.08 (-0.80 to 0.49)	0.95 (0.91 to 1.00)	-0.84 (-1.62 to -0.06)	138
	Dog	0.92	-3.01 (-5.25 to -0.80)	1.10 (1.00 to 1.20)	-0.65 (-1.91 to 0.62)	122
	Horse	0.95	0.00 (-1.47 to 0.00)	1.00 (1.00 to 1.09)	-0.02 (-0.72 to 0.69)	123
MCHC	Cat	0.67	8.58 (4.03 to 12.54)	0.67 (0.55 to 0.80)	-2.79 (-5.80 to 0.21)	138
	Dog	0.65	0.50 (-8.63 to 7.24)	1.00 (0.80 to 1.26)	0.65 (-1.80 to 3.11)	122
	Horse	0.50	8.42 (0.90 to 14.53)	0.79 (0.62 to 1.00)	0.75 (-1.51 to 3.02)	123
RDW	Cat	0.37	7.55 (4.19 to 10.17)	0.45 (0.33 to 0.59)	-5.19 (-10.24 to -0.15)	138
	Dog	0.73	4.54 (2.78 to 5.89)	0.56 (0.47 to 0.68)	-2.33 (-4.92 to 0.27)	121
	Horse	0.47	12.39 (10.00 to 14.41)	0.28 (0.19 to 0.38)	-4.98 (-8.83 to -1.13)	123
PLT	Cat	0.80	-9.47 (-52.65 to 23.06)	0.88 (0.74 to 1.05)	-38.3 (-225.5 to 149.0)	90
	Dog	0.97	-8.06 (-27.00 to 7.22)	1.15 (1.10 to 1.22)	42.5 (-73.9 to 158.8)	121
	Horse	0.84	-18.08 (-37.57 to 4.82)	1.04 (0.93 to 1.16)	1.3 (-82.3 to 84.9)	117
MPV	Dog	0.73	0.72 (-0.08 to 1.44)	0.69 (0.62 to 0.77)	-2.25 (-3.77 to -0.73)	106
	Horse	0.80	0.10 (-1.37 to 0.10)	1.00 (1.00 to 1.20)	0.10 (-0.50 to 0.69)	100

Table 3: ***Accuracy results for the differentiation for absolute numbers (#) and percentage (%) of the Mythic 18 compared with results from the Sysmex XT-2000iV and the manual differentiation***

Parameter	Species	Coefficient of correlation	Intercept (95% CI)	Slope (95% CI)	Bias (95% Limits of agreement)	Number of samples
LYM # (Sysmex)	Cat	0.52	0.09 (-0.51 to 0.54)	1.27 (1.00 to 1.63)	1.014 (-3.324 to 5.351)	115
	Dog	0.19	0.25 (-0.56 to 0.69)	1.11 (0.82 to 1.62)	0.687 (-2.683 to 4.056)	118
	Horse	0.89	0.16 (0.00 to 0.32)	0.96 (0.89 to 1.06)	0.127 (-0.957 to 1.212)	122
LYM # (Manual)	Cat	0.49	0.12 (-0.28 to 0.59)	1.50 (1.15 to 1.90)	1.348 (-3.280 to 5.975)	120
	Dog	0.14	0.42 (-0.25 to 0.91)	1.24 (0.88 to 1.86)	1.095 (-2.435 to 4.625)	117
	Horse	0.87	0.37 (0.16 to 0.55)	0.88 (0.80 to 0.97)	0.139 (-1.129 to 1.408)	123
LYM % (Sysmex)	Cat	0.79	6.38 (4.30 to 7.89)	0.92 (0.82 to 1.05)	6.23 (-12.93 to 25.40)	115
	Dog	0.62	7.91 (5.93 to 9.52)	0.60 (0.49 to 0.73)	2.07 (-14.46 to 18.60)	118
	Horse	0.88	2.75 (0.93 to 4.70)	0.97 (0.90 to 1.03)	1.55 (-10.92 to 14.02)	122
LYM % (Manual)	Cat	0.77	7.44 (4.85 to 9.92)	0.96 (0.84 to 1.11)	7.78 (-10.87 to 26.43)	120
	Dog	0.59	9.26 (7.72 to 11.06)	0.65 (0.52 to 0.81)	4.91 (-11.74 to 21.56)	117
	Horse	0.88	5.04 (2.74 to 6.83)	0.88 (0.81 to 0.96)	1.83 (-11.50 to 15.16)	123
MON # (Sysmex)	Cat	0.37	-0.05 (-0.19 to 0.05)	1.74 (1.33 to 2.31)	0.198 (-0.902 to 1.299)	117
	Dog	0.63	0.29 (0.19 to 0.38)	0.70 (0.57 to 0.86)	0.010 (-1.158 to 1.178)	119
	Horse	0.45	0.02 (-0.06 to 0.09)	0.77 (0.56 to 1.00)	-0.065 (-0.468 to 0.339)	122
MON # (Manual)	Cat	0.60	0.04 (-0.06 to 0.13)	1.43 (1.11 to 1.82)	0.253 (-0.860 to 1.366)	120
	Dog	0.57	0.34 (0.24 to 0.45)	0.63 (0.45 to 0.81)	0.047 (-1.366 to 1.460)	117
	Horse	0.24	0.15 (0.10 to 0.18)	0.61 (0.42 to 0.83)	0.074 (-0.402 to 0.550)	123
MON % (Sysmex)	Cat	0.10	1.34 (0.05 to 2.30)	0.98 (0.62 to 1.50)	0.83 (-8.12 to 9.79)	117
	Dog	0.16	1.69 (0.05 to 2.86)	0.84 (0.57 to 1.21)	0.31 (-7.24 to 7.86)	119
	Horse	0.28	0.18 (-0.92 to 1.19)	0.76 (0.53 to 1.06)	-0.91 (-5.96 to 4.14)	122
MON % (Manual)	Cat	0.00	2.00 (0.80 to 2.73)	0.82 (0.50 to 1.25)	1.45 (-4.76 to 7.65)	120
	Dog	0.24	3.56 (3.00 to 4.33)	0.44 (0.29 to 0.60)	0.39 (-7.22 to 8.00)	117
	Horse	0.04	1.90 (1.37 to 2.36)	0.60 (0.38 to 0.87)	0.79 (-5.73 to 7.30)	123
GRAN # (Sysmex)	Cat	0.97	0.52 (0.25 to 0.73)	0.83 (0.79 to 0.87)	-1.352 (-7.149 to 4.445)	115
	Dog	0.99	1.01 (0.72 to 1.29)	0.89 (0.86 to 0.93)	-0.451 (-5.235 to 4.332)	117
	Horse	0.98	0.24 (0.04 to 0.45)	0.93 (0.89 to 0.97)	-0.203 (-1.641 to 1.235)	122

GRAN # (Manual)	Cat	0.97	0.47 (0.19 to 0.82)	0.81 (0.77 to 0.85)	-1.643 (-7.967 to 4.682)	120
	Dog	0.99	1.05 (0.72 to 1.31)	0.86 (0.83 to 0.90)	-0.997 (-6.441 to 4.446)	117
	Horse	0.97	0.23 (0.03 to 0.52)	0.91 (0.86 to 0.95)	-0.347 (-2.013 to 1.318)	123
GRAN % (Sysmex)	Cat	0.74	-2.09 (-15.49 to 7.94)	0.96 (0.82 to 1.13)	-7.14 (-29.60 to 15.33)	115
	Dog	0.71	21.80 (12.05 to 30.00)	0.71 (0.59 to 0.82)	-2.55 (-18.84 to 13.75)	117
	Horse	0.84	2.76 (-3.18 to 8.07)	0.95 (0.87 to 1.04)	-0.65 (-16.14 to 14.85)	122
GRAN % (Manual)	Cat	0.74	-6.27 (-20.84 to 3.81)	0.99 (0.86 to 1.16)	-9.12 (-29.38 to 11.14)	120
	Dog	0.66	18.83 (6.55 to 28.97)	0.71 (0.59 to 0.86)	-5.33 (-22.39 to 11.72)	117
	Horse	0.84	3.90 (-2.50 to 10.72)	0.91 (0.82 to 1.00)	-2.61 (-18.06 to 12.84)	123

Furthermore, linear regression analysis by Passing-Bablok and Bland-Altman difference plots are presented in appendix Figure 3.1-3.12 (cat), Figure 4.1-4.13 (dog) and Figure 5.1-5.13 (horse).

The results of the Mythic 18 for RBC counts, HGB concentration, HCT and WBC counts (except for the cat), showed excellent correlation with the results provided by the reference instrument Sysmex XT-2000iV and manual HCT ($r \geq 0.98$). WBC results from the Mythic 18 were compared with the optical WBC results of the Sysmex XT-2000iV. Systematic errors with very small biases were observed in all three species for WBC counts. Generally, high WBC counts were underestimated by the instrument. The cat showed a very good correlation ($r = 0.94$), however the bias was small (-0.072). RBC counts showed an excellent result for dogs with a small bias due to a constant systemic error. For HGB levels a small proportional systemic error was seen in all investigated species. HCT values correlated excellently ($r = 0.99$) with the manual HCT results. Minor biases were observed in cats and horses due to a proportional error. Even in the dog, bias was less than 1%. MCV values showed excellent correlation in cat and dog and very good correlation in horses (r 0.94 to 0.96), with a systemic error and negative biases for horses and dogs. MCH results for horses showed an excellent Passing-Bablok regression line (Appendix Figure 5.9). The feline MCH showed an excellent correlation with a small negative bias due to a constant systematic error, whereas the dog showed a proportional systemic error with a negative bias. For MCHC a proportional systemic error was seen with biases from 0.65 g/dl in the canine samples, to -2.79 g/dl in the cat. For PLT counts, proportional systematic errors were observed in all three species with biases ranging from $1.3 \times 10^3/\mu l$ (horse) to $-38.3 \times 10^3/\mu l$ (cat), until $42.5 \times 10^3/\mu l$ for canine samples. In dogs, the Mythic 18 overestimated high PLT counts compared to the reference instrument. MPV results for feline samples were not available, because the Sysmex XT-2000iV determines PLT counts optically via flow cytometry.

The 3-part WBC differential showed for GRAN counts (absolute numbers) the best correlation and the smallest bias in all three species. LYM counts showed a strong positive bias in cats and dogs with wide 95% limits of agreement. In horses, correlation was found to

be good with a small bias. Results for MONO counts showed only fair correlation in canine samples and poor correlation in feline and equine samples. Some results of the WBC differential of the Sysmex XT-2000iV were excluded from statistical analysis due to the inability of the Sysmex XT-2000iV to differentiate WBC: one equine, two canine and four feline blood samples. In the equine sample, both canine samples and three of the four feline samples, the Sysmex XT-2000iV misclassified a left shift. In the remaining feline sample normoblasts (52 normoblasts per 100 WBC) were seen in the blood smear while the Sysmex XT-2000iV classified them falsely to LYM.

The accuracy results of the microscopic WBC differential and the WBC differential provided by the Sysmex XT-2000iV are shown in Table 4.

Table 4: ***Accuracy results of the differentiation for absolute numbers (#) and percentage (%) of the Sysmex XT-2000iV and the manual WBC differentiation***

Parameter	Species	Coefficient of correlation	Bias (95% limits of agreement)	Number of samples
LYM #	Cat	0.83	0.222 (-2.105 to 2.549)	114
	Dog	0.86	0.355 (-0.966 to 1.675)	115
	Horse	0.91	0.032 (-0.999 to 1.062)	123
LYM %	Cat	0.79	2.2 (-18.47 to 22.87)	114
	Dog	0.92	2.55 (-5.50 to 10.59)	115
	Horse	0.88	0.61 (-13.21 to 14.42)	123
MONO #	Cat	0.49	0.082 (-0.840 to 1.004)	115
	Dog	0.83	0.066 (-0.898 to 1.030)	116
	Horse	0.54	0.158 (-0.340 to 0.657)	123
MONO %	Cat	0.52	1.15 (-10.24 to 12.54)	115
	Dog	0.78	0.37 (-4.72 to 5.45)	116
	Horse	0.58	2.01 (-4.70 to 8.71)	123
GRAN #	Cat	0.99	-0.317 (-3.154 to 2.521)	114
	Dog	1.00	-0.540 (-2.731 to 1.651)	114
	Horse	0.98	-0.196 (-1.433 to 1.041)	123
GRAN %	Cat	0.70	-3.27 (-31.39 to 24.86)	114
	Dog	0.93	-2.95 (-11.62 to 5.72)	114
	Horse	0.82	-2.60 (-20.17 to 14.97)	123

4.2 Precision

Coefficients of variation from the precision study in series (Table 5) ranged from 0% for normal and low monocyte counts in cats and dogs to 34.91% for low monocyte counts in cats (0.1-0.2x10³/µl). RBC, HGB, HCT and Indices had CVs <1.7%. WBC counts had CVs <2%, except of the feline and equine sample with low WBC. For PLT counts >200x10³/µl CVs ranged from 3.17% to 4.67%, for platelets <200x10³/µl from 5.65% to 10.24% in a horse sample.

Table 5: ***Precision in series: mean values and coefficients of variation for blood samples from cats, dogs and horses with low (L), normal (N) and high (H) values for total WBC count***

Parameter	Species	L		N		H	
		Mean	CV	Mean	CV	Mean	CV
WBC (x10³/µl)	Cat	1.96	2.50	6.81	1.50	40.92	1.17
	Dog	2.99	1.92	6.77	1.68	80.71	0.98
	Horse	1.72	3.24	7.85	1.58	19.76	0.95
LYM (x10³/µl)	Cat	1.21	4.75	1.87	3.06	7.08	6.12
	Dog	1.05	4.74	1.64	5.81	9.75	3.93
	Horse	0.69	9.32	1.96	4.13	3.27	4.07
MONO (x10³/µl)	Cat	0.13	34.91	0.2	0	3.58	10.16
	Dog	0.2	0	0.8	6.45	3.12	4.25
	Horse	0.09	27.74	0.21	12.43	0.46	13.14
GRAN (x10³/µl)	Cat	0.6	8.61	4.75	1.7	30.25	3.93
	Dog	1.77	3.23	4.33	4.17	67.84	1.34
	Horse	0.99	2.59	5.66	1.84	16.05	1.88
LYM (%)	Cat	62.01	2.94	27.57	2.33	17.32	7.04
	Dog	34.75	3.89	24.33	5.86	12.08	4.00
	Horse	39.61	4.11	25.02	3.17	16.49	4.41
MONO (%)	Cat	7.29	6.00	2.85	8.38	8.78	10.79
	Dog	5.98	8.81	11.69	6.94	3.87	4.26
	Horse	3.81	12.91	2.82	8.49	2.31	9.07
GRAN (%)	Cat	30.7	6.11	69.58	0.83	73.9	2.88
	Dog	59.27	2.30	63.98	3.21	84.05	0.68
	Horse	56.57	2.76	72.16	1.28	81.19	1.13
RBC (x10⁶/µl)	Cat	6.96	1.30	9.17	0.72	6.04	0.94
	Dog	3.99	0.75	7.85	0.54	4.87	1.32
	Horse	6.25	1.18	8.85	1.01	11.48	0.78
HGB (g/dl)	Cat	8.28	0.79	12.91	0.53	7.09	0.62
	Dog	8.65	0.93	15.79	0.56	9.99	1.03
	Horse	11.49	1.13	15.06	0.85	19.73	0.45
HCT (%)	Cat	26.38	1.66	39.51	0.82	26.21	1.02
	Dog	26.50	0.86	46.36	0.68	31.14	1.40
	Horse	30.17	1.47	40.43	0.95	51.91	1.13
MCV (fl)	Cat	37.93	0.67	43.09	0.31	43.39	0.58
	Dog	66.37	0.34	59.05	0.43	63.92	0.43
	Horse	48.25	0.70	45.66	0.28	45.20	0.49

MCH (pg)	Cat	11.90	1.15	14.07	1.02	11.74	0.81
	Dog	21.68	0.97	20.11	0.83	20.49	1.08
	Horse	18.36	0.86	17.02	0.87	17.21	0.60
MCHC (g/dl)	Cat	31.40	1.36	32.67	1.07	27.06	1.05
	Dog	32.67	1.19	34.06	1.06	32.08	1.32
	Horse	38.09	1.37	37.26	0.90	38.01	0.96
RDW (%)	Cat	21.93	1.74	16.88	1.90	16.99	2.10
	Dog	16.32	2.60	16.06	2.27	13.23	1.60
	Horse	18.61	2.13	18.46	2.81	18.68	1.52
PLT ($x10^3/\mu l$)	Cat	133.47	5.65	249.73	4.63	170.07	6.91
	Dog	305.73	3.17	215.47	3.98	144.53	6.23
	Horse	105.79	10.24	205.36	4.67	73.21	7.66
MPV (fl)	Cat	9.01	2.68	9.65	0.99	9.72	2.10
	Dog	9.38	1.24	7.50	1.89	10.12	2.11
	Horse	8.01	1.97	7.69	2.08	6.94	3.52

Table 6 shows the results from day-to-day precision analysis. CVs ranged from 0.6% for MCV values to 20.1% for low MONO counts. For RBC, HCT, HGB and Indices, CVs were <2.3%. WBC counts had CVs from 0.8% (18.8-19.3x10^3/µl) to 3.2% (1.9-2.1x10^3/µl). For PLT counts, CVs ranged from 4.6% (443-529x10^3/µl) to 8.4% (69-95x10^3/µl).

Table 6: ***Precision from day to day: mean value and coefficient of variation for control blood samples***

Parameter	Low		Middle		High	
	Mean	CV	Mean	CV	Mean	CV
WBC ($x10^3/\mu l$)	2	3.2	7.4	2	19	0.8
LYM ($x10^3/\mu l$)	1.1	5.7	2.1	5.1	3.1	6.6
LYM (%)	55.1	3.3	29.1	3.7	16	5.8
MON ($x10^3/\mu l$)	0.2	20.1	0.4	9.4	0.6	3.6
MON (%)	11.7	8.5	5.8	5.7	3.2	5
GRAN ($x10^3/\mu l$)	0.7	7.9	4.8	2	15.4	1.2
GRAN (%)	33.2	3.7	65.1	1.7	80.8	1.3
RBC ($x10^6/\mu l$)	2.57	1.9	4.97	1.6	5.99	1.2
HGB (g/dl)	6.6	2.2	14.4	1.5	18.8	1.4
HCT (%)	17	2	36.9	1.2	48	1
MCV (fl)	66.2	0.8	74.2	0.7	80.2	0.6
MCH (pg)	25.6	1.9	28.9	1.6	31.4	1.3
MCHC (g/dl)	38.7	1.9	39	1.4	39.1	1.3
RDW (%)	16.8	3.4	16.2	2.9	14.2	2.7
PLT ($x10^3/\mu l$)	84	8.4	236	5.9	482	4.6
MPV (fl)	8.4	3.7	8	2.3	7.8	2

4.3 Linearity

Results of the linearity study are presented in Table 7. Linearity plots are shown in the appendix Figure 6 (cat), Figure 7 (dog) and Figure 8 (horse). For all tested parameters the instrument demonstrated good linearity. The tested ranges of linearity were within the

ranges provided by the manufacturer for human blood except for canine WBC counts. The linearity ranges were up to 100x10³/µl for feline WBC and 95x10³/µl for canine sample. RBC showed linearity until 11.4x10⁶/µl in canine, 12.6x10⁶/µl in equine and 14x10⁶/µl in feline sample. HGB was linear over the measured range and HCT up to 62% in a cat, 70% in a horse and 71% in a dog. Linearity study with platelet enriched plasma from a horse showed PLT linearity over the measurement range until 1020x10³/ µl.

Table 7: ***Range of linearity for canine and feline WBC, RBC parameter and equine PLT in cats, dogs and horses and for human blood samples from Orphée***

Species	Parameter	Range of linearity
Cat	WBC	-100 (x10³/µl)
	RBC	-14 (x10⁶/µl)
	HGB	-23.2 (g/dl)
	HCT	-70 (%)
Dog	WBC	-95 (x10³/µl)
	RBC	-11.4 (x10⁶/µl)
	HGB	-24.3 (g/dl)
	HCT	-71 (%)
Horse	RBC	-12.6 (x10⁶/µl)
	HGB	-24.4 (g/dl)
	HCT	-62 (%)
	PLT*	-1.080 (x10³/µl)
Range provided by Mythic for human blood	WBC	0-100 (x10³/µl)
	RBC	0.1-8 (x10⁶/µl)
	HGB	0.5-24 (g/dl)
	HCT	5-70 (%)
	PLT	5-2.000 (x10³/µl)

* For this study Platelet enriched plasma from a horse was used

4.4 Carry-over

Table 8 presents the results of the carry-over experiment. Each sample was measured twice ("value" in Table 8 represents the result of the second sample analysis), followed by three diluent measurements. Carry-over is the percentage of cells that were measured in the first diluent analysis. The results of the second and third diluent analyses were always zero. All results for carry-over lie in the range provided by the manufacturer (<1%), except one sample for feline WBC counts.

Table 8: ***Results for carry-over of the Mythic 18 for WBC, RBC and PLT***

Parameter	Species	Value	Carry-over %	Value	Carry-over %
WBC ($x10^3/\mu l$)	Cat	45.8	0.22	9.0	1.11
	Dog	40.1	0.25	5	0
	Horse	9.4	0	5.5	0
RBC ($x10^6/\mu l$)	Cat	3.76	0.26	7.14	0.56
	Dog	5.31	0.19	6.87	0.15
	Horse	7.9	0.25	8.05	0.25
HGB (g/dl)	Cat	4.2	0	10.4	0
	Dog	12.2	0	13.4	0
	Horse	14.4	0	13.8	0
PLT ($x10^3/\mu l$)	Cat	620	0.97	579	0
	Dog	336	0	146	0
	Horse	147	0	147	0

4.5 Cell aging

Table 9 shows the results of the cell aging study. First significant changes appeared after 6h for HGB in the feline samples, but not at the following time points. In canine blood samples a significant change in RBC counts was detected after 24h, not at the following time points. Feline MCHC showed significant changes at time point 10h, 24h, 32h and 48h showing a decrease; in horses also at time point 32h and 48h. After 24h, equine WBC values started to decrease significantly, and the LYM-GRAN ratio moved in favour of LYM count. Canine and feline samples presented significant changes for MCV values after 24h, 32h and 48h showing an increase. At time point 48h feline HCT values and canine MCH values and MONO% started to increase statistically significant.

Table 9: ***Statistically significant changes in blood cell parameters in a cell aging study over two days***

Species	Parameter	6 h	10 h	24 h	32 h	48 h
Cat	MCHC	-	(5.8%) ↓			
	MCV	-	-	(8.6%) ↑		
	HCT	-	-	-	-	(16.1%) ↑
	HGB	*(3.5%) ↓	-	-	-	-
Dog	RBC	-	-	*(4.8%) ↓	-	-
	MCV	-	-	(5.2%) ↑		
	MCH	-	-	-	-	(6.4%) ↑
	MONO %	-	-	-	-	(39.7%) ↑
Horse	WBC	-	-	(6.6%) ↓		
	LYM	-	-	(118%) ↑		
	GRAN	-	-	(49.2%) ↓		
	MCHC	-	-	-	(3.8%) ↓	

* Significant change only one-time - no significant change

4.6 General Performance of the Mythic 18

Mythic 18 was found to be a well-designed and user-friendly instrument, which was easy to handle and could be operated after a short instruction. Results of the quality control analysis are provided as readily cumulative results over time enabling the user to review and compare them comfortably. General care and maintenance of the instrument during the evaluation period was easy and quickly done. For daily work in the morning a start-up procedure and in the evening a cleaning step prior to the shutdown was done. In case of increased WBC counts appearing during the blank measurement in the start-up menu, or after the analysis of blood samples with WBC counts higher than 100x10³/µl, the instrument has to be bleached with 4 ml of a Sodium hypochlorite solution (>10%) which has to be added to both counting chambers. Samples with very high WBC counts occasionally lead to clogging of the orifice which may lead to a decreased measurement volume in the following samples.

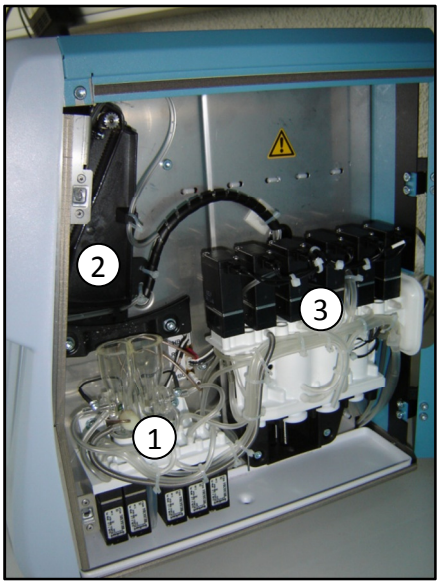


***Figure 2*: Three modules of the Mythic 18**

The user can open the side door on the right side where the counting chamber (1), syringe (2) and sampling module (3) can be inspected (Figure 2). On the left side of the instrument the M-pack (reagents) are integrated in the instrument. Prior to analysis, the user can select the animal species directly via the touch screen; exhibiting symbols for dog, cat, horse and other species. Analysing time per blood sample is around 1 minute allowing very short turn-around-times for veterinarians and patients.

4.7 Clinical relevance

Some of the results deviate from those determined by the reference methods. In Table 10 the number of results that deviate and their clinical relevance are compiled.

Table 10: ***Clinical relevance of the Mythic 18 results that deviate from those of the reference methods***

Parameter	Species	Correctly recognized samples		Not correctly recognized samples		Number of samples
		< reference range (<)	> reference range (>)	False positive (<)	False positive (>)	
WBC	Cat	1/4	39/48	3	9	129
	Dog	2/2	60/61	-	3	122
	Horse	9/9	50/53	-	3	123
LYM (absolute)	Cat	11/31	3/5	-	18	129
	Dog	6/24	6/8	5	18	122
	Horse	8/13	14/16	1	6	123
GRAN (absolute)	Cat	2/3	30/40	2	3	129
	Dog	-	57/58	-	2	122
	Horse	5/6	6/8	2	1	123

RBC	Cat	32/35	7/7	-	-	129
	Dog	45/51	7/8	-	2	122
	Horse	18/20	19/20	2	-	123
HCT	Cat	54/58	3/3	2	-	126
	Dog	59/60	2/4	4	-	121
	Horse	21/21	10/12	4	-	123
PLT	Cat	19/24	1/1	13	-	90
	Dog	13/17	22/22	-	8	121
	Horse	9/12	15/20	9	11	122

Table 11 presents the pathologies which were seen in the blood smear during manual WBC differentiation.

Table 11: ***List of samples where pathologies were missed by the Mythic 18***

Missed pathologies	Cat	Dog	Horse
Left shift	15/129	29/117	9/123
Normoblasts	12/129	27/122	2/123
Reactive Lymphocytes	33/123	10/117	32/123
Foamy basophilia (NEUTROPHILS)	14/123	19/117	21/123
Atypic LYM	-	1	-
Platelet aggregation	48/90	11/121	24/117
Large platelets	7/90	20/121	-

5. Discussion

Most in-house haematology analysers used in veterinary diagnostics were manufactured for human medical purposes (Bleul et al., 2002). Considerable differences in blood cell sizes and WBC morphology between human and animal blood as well as among different animal species require modification of the instruments software. For the Mythic 18, specifications for canine, feline and equine blood samples have been developed in cooperation with the manufacturer in the Clinical laboratory, Vetsuisse-Faculty, University of Zurich. Briefly, for each investigated species, gains and thresholds for RBC, WBC and PLT have been adopted. Furthermore, the quantity and exposure time of the lysis reagent were determined (Weiser, 1987b). Correction factors were defined for WBC, RBC, HGB, HCT, PLT and MPV. To confirm the accuracy of these species specific settings, this evaluation study was conducted by comparing the results of the Mythic 18 with a reference instrument and manual methods.

In the present study, the Sysmex XT-2000iV was used as reference instrument. This haematology analyser is widely used in large and referral veterinary clinical laboratories and has been validated for its use in cats, dogs and horses (Lilliehöök and Tvedten, 2009a, b; Weissenbacher et al., (2010)). Manual chamber counting of WBC, RBC and PLT, are well-known as gold standard techniques. However, these methods show high imprecision due to the limited quantity of counted cells, artefacts, and classification of the cells (Kjelgaard-Hansen and Jensen, 2006; Knoll and Rowell, 1996; Lilliehöök and Tvedten, 2009b). Therefore,

electronically blood cell counting methods have mainly replaced the former gold standard techniques. Microscopic WBC differentiation of a blood smear is still mandatory to confirm WBC abnormalities and to rule out the presence of platelet clumps, RBC parasites and blood cell precursors. Nevertheless, this manual technique is prone to high imprecision, especially for those cells that are underrepresented in the blood (Weissenbacher et al., (2010)). Therefore, this study is based on the comparison of the 3-part WBC differential of the Mythic 18 with both, manual and electronically WBC differentiation.

Results for RBC parameter of the Mythic 18 showed very good to excellent agreement with the Sysmex XT-2000iV results, except for MCHC and RDW. RBC in the dog showed a perfect slope and a small constant systemic error yielding to the negative biases for MCH and MCV. A small adaption of the correction factor of the RBC could improve the results. Canine MCV values of the Mythic 18 showed a small negative bias compared to the Sysmex XT-2000iV which would lead to different clinical conclusion in some cases. Small changes of the HCT correction factor in the canine settings could improve MCV agreement between the Sysmex XT-2000iV and the Mythic 18. Otherwise, adjustment of the reference limits for canine MCV would be indicated. Difference in the osmolarity of the diluents between the Sysmex XT-2000iV (250 mosm/kg) and the Mythic 18 (332 mosm/kg) can possibly cause the negative bias in canine MCV values. The hypotonic diluent of the Sysmex causes swelling of the RBC whereas the relatively isotonic diluent of the Mythic produces comparatively lower MCV values (Boisvert et al., 1999). The agreement for MCHC values is less satisfactory in all three evaluated species. Low correlation for this parameter has been reported in previous studies (Sanzari et al., 1998; Weissenbacher et al., (2010); Wenger-Riggenbach et al., 2006), and can be mainly explained by the narrow concentration range of this parameter. In feline and equine samples, the HCT values showed nearly no bias. This excellent agreement can be mainly attributed to the fact that the HCT of the Mythic 18 has been calibrated to the manual HCT in the same laboratory and with the same equipment as the evaluation study was performed.

Total WBC count agreed excellently in horses and dogs compared to the optical WBC counts of the reference instrument. Generally, the Mythic 18 underestimated high total WBC counts in all three species on average by a few percentage points. In the feline samples, total WBC correlation is very good. However, in 23 out of 129 feline samples, the WBC were on average more than 1000 WBC/µl higher than those of the reference method in which the WBC are determined by an optical flow cytometry principle (Knoll, 2000) (Appendix Figure 3.1). This can readily be explained by the fact that feline PLT have a high tendency to aggregate and that the aggregates are counted as WBC. When 2 samples with the extremely high overestimation of more than 15.000 WBC/µl were removed from the statistic as outliers, the coefficient of correlation improved to 0.97, and the bias decreased from -0.043 to -0.364. In all 23 samples, platelet aggregates could be identified in the blood smear. It is a well-known phenomenon in cats that platelet clumps or large platelets can cause falsely increased WBC count and decreased PLT counts in impedance-based haematological instruments (Knoll and Rowell, 1996; Norman et al., 2001). During a software adaption of the

Mythic 18, a lower correction factor for total WBC count was chosen to counterbalance feline samples with overestimated WBC counts due to platelet aggregates. This finding highly supports the usefulness of instrument evaluation studies, in which analysers using different technologies are compared against each other.

In horses, cats and dogs GRAN are predominant in the blood (Table 1), therefore imprecision for this leukocyte subtype is low, and the Mythic 18 showed excellent agreement with both, the Sysmex XT-2000iV and the manual WBC differentiation (Roleff et al., 2007). Precision and accuracy in canine and feline LYM counts in the Mythic 18 were not satisfactory. This finding has already been demonstrated for both species in the VetScan HMT (Dewhurst et al., 2003), for canine samples in the CA530-Vet (Roleff et al., 2007) and the Heska CBC (Becker, 2007). As LYM count in horses showed good agreement, the Mythic 18 can be judged as reliable for counting LYM in horses. A recent evaluation of impedance based haematology instruments with equine blood samples showed also good agreement for the WBC with manual techniques, however LYM counts were slightly underestimated (Deprez et al., 2009).

PLT counts of the Mythic 18 showed excellent agreement in the dog and good agreement in cat and horse with the Sysmex XT-2000iV. Compared to previous studies of impedance based haematology instruments, the Mythic 18 showed better agreement with the reference methods for cats, dogs and horses (Becker, 2007; Deprez et al., 2009). Canine PLT counts obtained by the Heska CBC, the Scil Vet ABC and the VetScan HMT displayed lower agreement and negative biases. For feline PLT counts the Heska CBC had little bias but large random error, whereas the Scil Vet ABC and the VetScan HMT showed similar results as the Mythic 18 know ever with a lower precision. The good results for feline PLT counts were remarkable, particularly as samples with platelet aggregates were included in the calculation. Impedance based haematology instruments normally have problems in counting feline PLT accurately (Norman et al., 2001). PLT and RBC sizes in cats often overlap (Zelmanovic and Hetherington, 1998) and impedance based instruments differentiate cells based on their size. In the present study more than 53% of the feline samples showed platelet aggregation. The good results in this study can be mainly explained by the excellent adaption of the feline threshold setting. Despite the apparently good capability of the Mythic 18 in counting feline PLT, it is highly recommended to screen a blood smear of each feline sample of the presence of platelet aggregates. This is also indicated to verify the reliability of WBC count of the Mythic 18. Additionally, intensive mixing of feline blood samples is known to decrease the amount of platelet aggregates (Tvedten and Korcal, 2001).

MPV values showed good agreement only for horses, because the correction factor has been adapted. The Mythic 18 provides MPV values for cats. In other instruments this parameter is usually not reported (Zelmanovic and Hetherington, 1998). However, the Sysmex XT-2000iV did not provide MPV values for the cat, due to the fact that the feline PLT were measured in the optical channel of the instrument. Therefore, no conclusion can be drawn about the results of the feline MPV values of the Mythic 18.

The Mythic 18 showed excellent results for the precision analysis. Lower values usually present a higher variation. Generally, PLT counts and the 3-part WBC differentiation showed higher variation. Higher variations were also caused by the fact that the instrument releases only one decimal place per parameter, except for RBC counts.

Results for the linearity study showed that the Mythic 18 underestimated high WBC values and high HCT values. This is not of severe clinical relevance, as these values were far above the upper reference limit. RBC, HGB and PLT in the platelet enriched plasma demonstrated excellent linearity.

Carry-over of the Mythic 18 is negligible and should have no clinically relevant influence of the following sample.

The Mythic 18 was built with aim to be an in-house haematology analyser for veterinary practitioners. Generally under practice conditions sample analysis is done immediately after blood collection. For horses all values were stable 24 hours. Longer storage would lead to underestimation of WBC counts and WBC differentiation would show falsely elevated LYM count and falsely decreased GRAN count. In cats only RBC parameters were compared, because formation and disaggregation of platelet clumps seemed to be time-depending inducing remarkable changes in WBC and PLT counts over time (unpublished observation).

5.1 Clinical relevance of the results

In the majority of samples analysed, results of the Mythic 18 would have led to the same clinical interpretation as the results obtained by the reference methods.

Total WBC results in cats reflect the phenomenon that the Mythic 18 overestimated total WBC counts when platelet aggregation is present in the sample. Only in one of four (25%) feline cases leukopenia was detected by the Mythic 18. In two of the three samples where the Mythic 18 did not recognize the leukopenia, platelet aggregates were identified in the blood smear. In the remaining sample large platelets were found. The three erroneous samples with leukopenia showed differences of 0.67-1.15x10^3/µl WBC. Leukocytosis was detected correctly in 39 of 48 (81.3%) cases. False positive leukocytosis was found in 9 of all 129 (7%) feline samples, and was caused by platelet clumps. In dogs, leukocytosis was correctly identified in 60 out of 61 (98.4%) blood samples. In two of the three samples where WBC were falsely counted as high, differences in values between the Mythic 18 and the Sysmex XT-2000iV were 38% and 67%, because of large platelets and platelet clumps. Equine and canine leukopenia was correctly identified in all investigated cases. Leukocytosis in horses was correctly recognized in 50 of 53 (94.3%) cases. One of three samples where WBC were falsely counted as high showed a 45% difference in values due to platelet aggregations. For feline LYM, the Mythic 18 identified 11 of 31 (35.5%) samples with lymphocytopenia correctly. In the remaining 20 feline cases with lymphocytopenia, the Mythic 18 overestimated LYM count due to the presence of platelet aggregates or large platelets. In all cases where the Mythic 18 revealed a lymphocytopenic cell count result, the result was

accurate. This does not exclude that occasionally a lymphocytopenia may not be detected, if more samples had been tested. Three out of 5 (60%) feline samples having lymphocytosis were correctly identified by the Mythic 18. However, in 18 feline samples the results of the Mythic 18 would have led to a false result of lymphocytosis. Again, this can be explained by the fact that the instrument counts platelet aggregates or large platelets in most of the cases as lymphocytes. In the dog, only 6 out of 24 (25%) lymphocytopenic blood samples were identified correctly. Additionally, 5 samples were falsely characterized as lymphocytopenic although the values were in the reference range. Furthermore, 6 out of 8 (75%) samples with lymphocytosis were correctly identified, whereas 18 samples (14.8% of the 122 samples) with normal LYM count were identified as having lymphocytosis. For this high degree of misclassification in the LYM count of the dog, no obvious explanation can be provided.

In the cat, 2 out of 3 (66.7%) granulocytopenic blood samples were correctly identified by the Mythic 18. The only misidentified sample showed only a slight difference (7%) and would not have led to a different clinical conclusion. Granulocytosis in the cat was correctly identified in 30 of 40 cases (75%). In the remaining cases, the Mythic 18 had lower total WBC counts compared to the Sysmex XT-2000iV. In 2 of 10 of the cases, the clinical interpretation would have been different. Platelet aggregates had led to overestimated WBC counts in three feline samples, as described above and therefore to false positive GRAN counts. In the dog, the Mythic 18 identified 57 out of 58 (98%) samples with granulocytosis correctly. The ability of the Mythic 18 of detecting granulocytopenic samples in dogs cannot be judged, as during this study no samples with granulocytopenia were submitted for analysis. In horses the Mythic 18 identified correctly granulocytopenia in 5 out of 6 (83%) of the cases and granulocytosis in 6 out of 8 (75%) of the equine cases. False positive granulocytosis and granulocytopenia was mainly due to differences in total WBC counts between the Mythic 18 and the reference instrument.

In most of the cases, RBC results from the Mythic 18 would have lead to the same clinical decision than those of the reference instrument. Differences in canine samples in relation to the reference range were all below 10%. One out of two equine samples falsely showed anaemia with underestimation of 10.2% for the Mythic 18 value compared to the Sysmex XT-2000iV. One feline sample did not recognized anaemia with a result 23.1% higher than the Sysmex XT-2000iV result. No explanation can be offered for this discrepancy, however no different clinical conclusion would have been drawn from this result. For equine HCT values, 4 false positive samples assuming anaemia occurred without any impact on the clinical decision. As the differences were less than 2%, which is attributed to the imprecision of the HCT reading in the capillary tube.

In 19 of 90 cat samples the Mythic 18 and the Sysmex XT-2000iV showed thrombocytopenia. Five feline samples with thrombocytopenia were detected only by the Sysmex XT-2000iV. Four out of these 5 feline blood samples demonstrated moderate to severe platelet aggregation in the blood smear. Additionally, in 13 feline samples, the Mythic 18 falsely showed a thrombocytopenia. Platelet aggregation was only found in one of these 13 cases, and giant platelets in 3 cases. In the remaining cases no explanation for the detection of false positive thrombocytopenia can be offered. It has been demonstrated, that EDTA

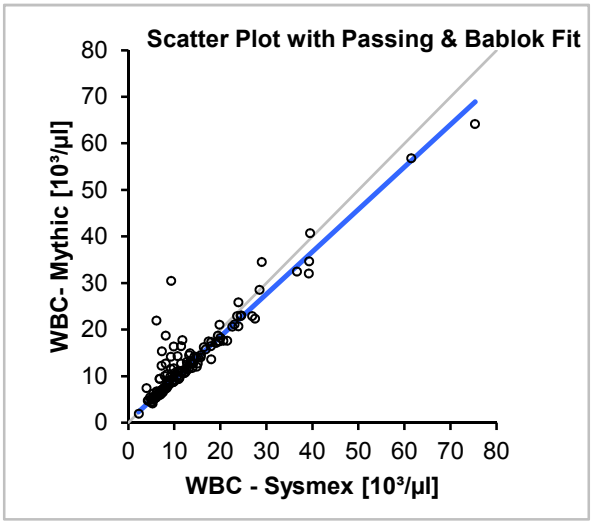
anticoagulated blood is prone to build platelet aggregates in cats (Moritz and Hoffmann, 1997). Aggregation of feline platelet seems to occur time dependent and spontaneously (manuscript in preparation). Thrombocytosis in the dog was detected by the Mythic 18 in 22 out of 22 (100%) of the cases. The samples were the Mythic 18 falsely showed thrombocytosis can be explained by the fact that the Mythic 18 overestimated PLT counts in the higher range. In 13 out of 17 (72%) of the cases, thrombocytopenia was correctly identified in the dog. The 5 samples where the Mythic 18 missed thrombocytopenias can be attributed to the positive bias of the Mythic 18 for PLT counts. Four of these samples showed PLT counts with average of $70x10^3/\mu l$ PLT higher than the Sysmex XT-2000iV results, this could have lead to a different clinical interpretation. One sample showed a slight difference of 2%. In the equine samples erroneous thrombocytopenia was identified by the Mythic 18 in 7.4% (9 of 122) of the cases. One sample showed platelet aggregates, in the remaining cases random error is the most likely explanation for the deviation. Different clinical conclusions could be drawn in one sample where the Mythic 18 showed thrombocytopenia instead of normal PLT counts identified by the Sysmex XT-2000iV, and in two samples where the instrument measured PLT counts within the reference limits instead of identifying thrombocytopenia. High CVs in the equine precision study as well as the narrow range of reference limits may contribute to the high rate of misclassification in equine PLT counts.

In the present study, important pathologies would have been missed, when relaying only on the electronically WBC differential of the Mythic 18 (Table 11). Two canine samples showed more than 4 nucleated red blood cells, while one feline sample showed 52 normoblasts. In these samples, WBC results were falsely increased, and would have led to different clinical conclusions. One canine sample presented atypical LYM due to an immune mediated disease. Left shifts and especially degenerative left shifts would have been missed in a remarkable number of blood samples in the canine and feline samples. Foamy cytoplasm of segmented neutrophils has been observed in all three species by manual microscopy. This is an important morphological indicator for severe inflammatory disease and toxicity. The presence of reactive LYM is a useful hint to antigenic stimulation in the patient (Stockham and Scott, 2008). All this changes give important information to the clinician and help to improve patient care.

6. Conclusion

The Mythic 18 was found to perform very well for RBC parameters and total WBC counts in all investigated species. In cats it is important to ensure that no platelet aggregates are presented, otherwise WBC and platelets values should be determined by manual methods. GRAN and LYM counts are accurate in horses. In dogs and cats absolute granulocyte counts are reliable. As with all impedance based haematological instruments, a microscopic blood smear evaluation is indicated to identify platelet aggregates, normoblasts, left shift, cell precursors and blood parasites and to verify WBC differentiation. Flags for pathological values and reference limits need to be created by the manufacture of the instrument.

7. Appendix



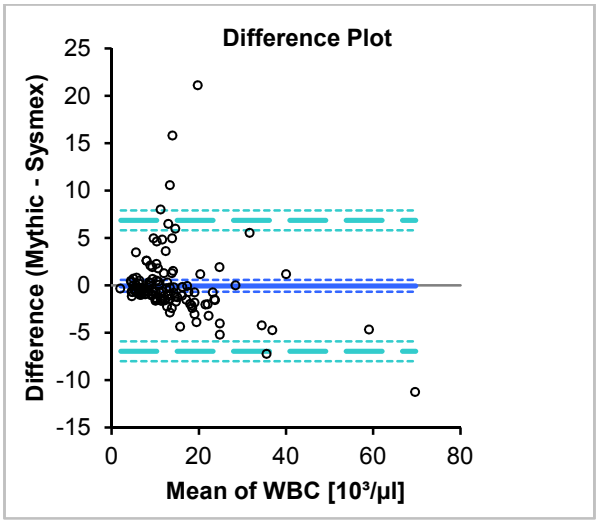
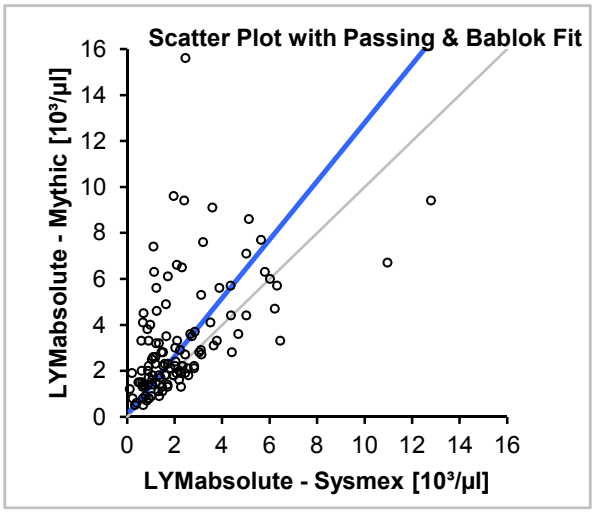


***Figure 3.1*: feline WBC**



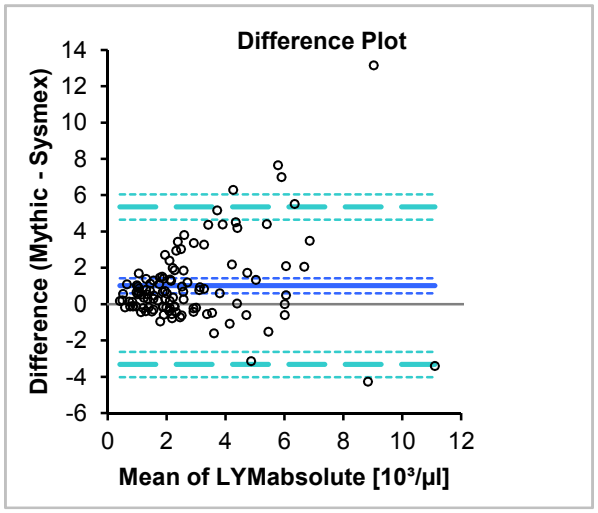
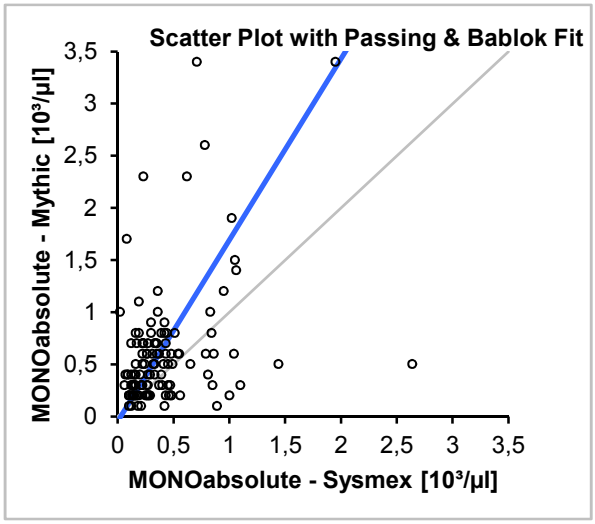


***Figure 3.2*: feline LYM #**



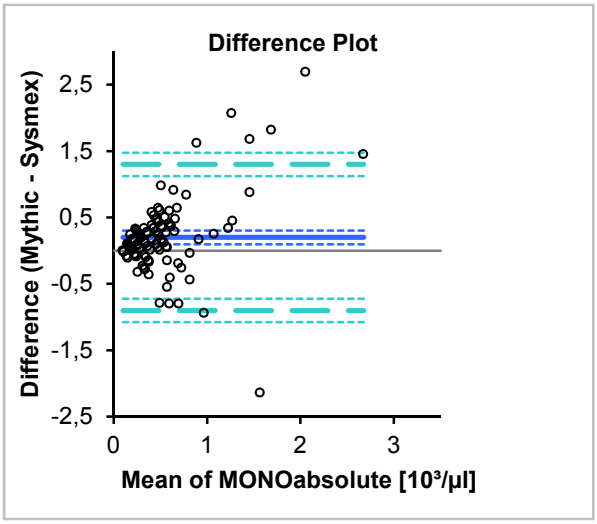
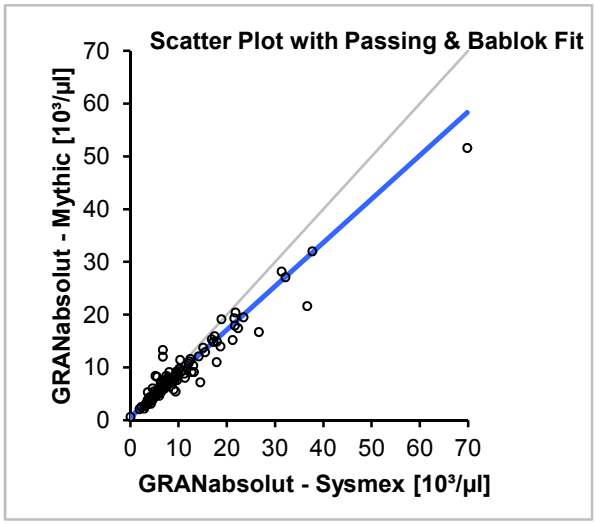


***Figure 3.3*: feline MONO #**



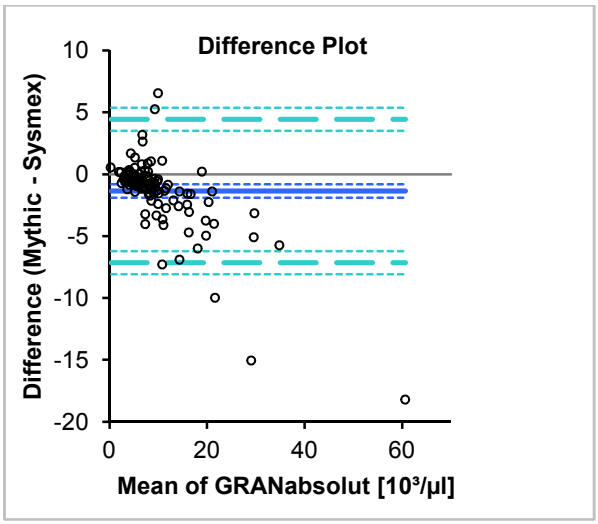
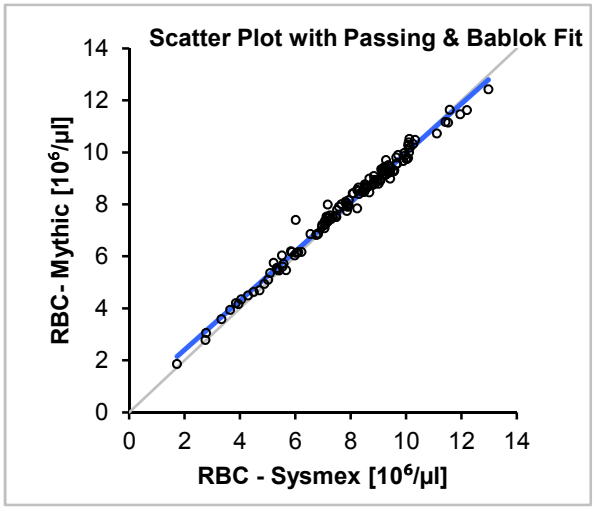


***Figure 3.4*: feline GRAN #**



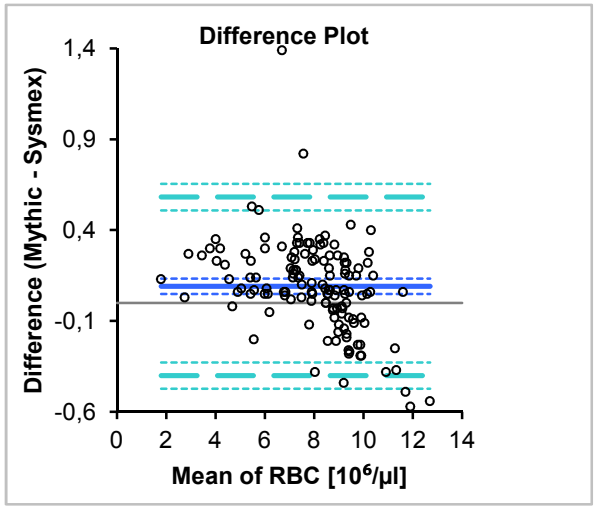
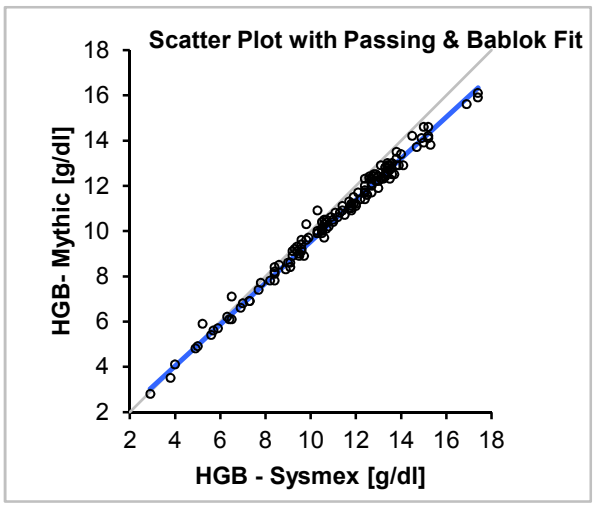
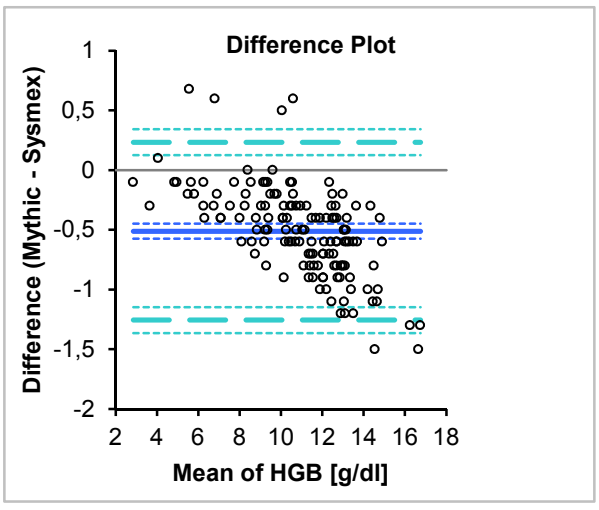


***Figure 3.5*: feline RBC**





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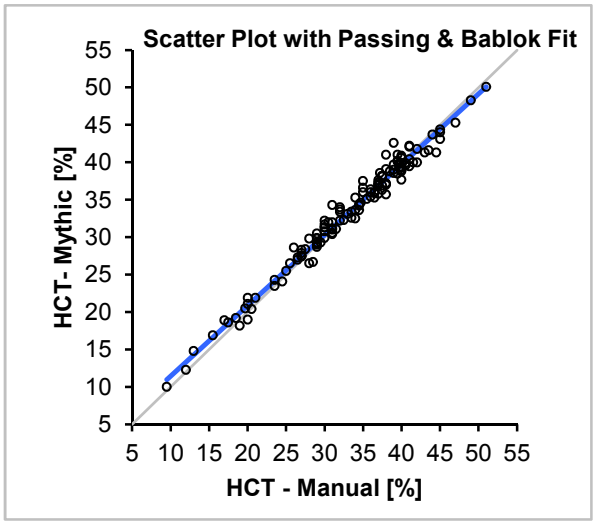


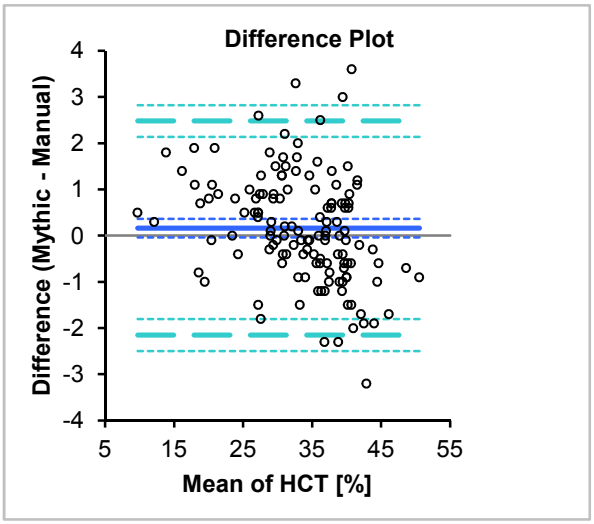
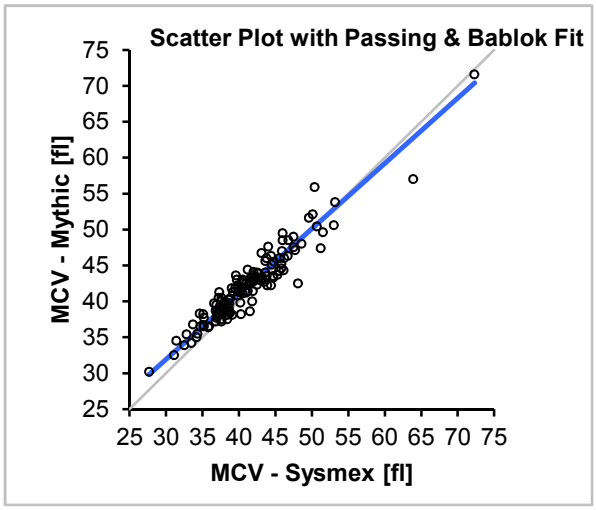


***Figure 3.7*: feline HCT**



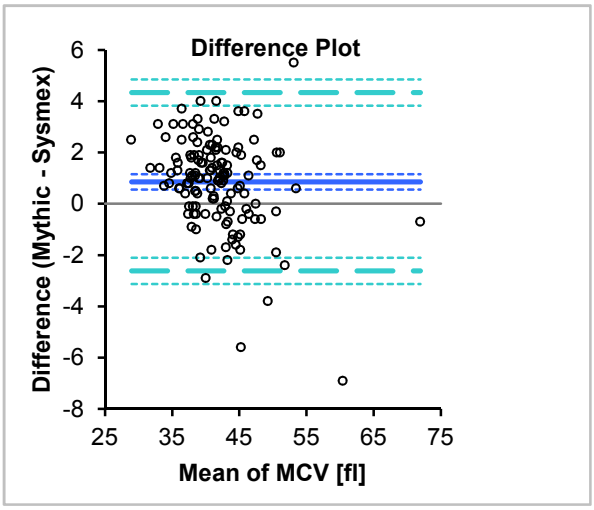
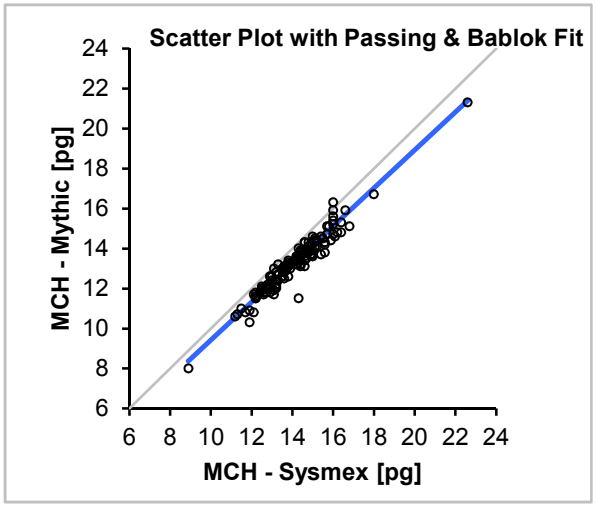


***Figure 3.8*: feline MCV**



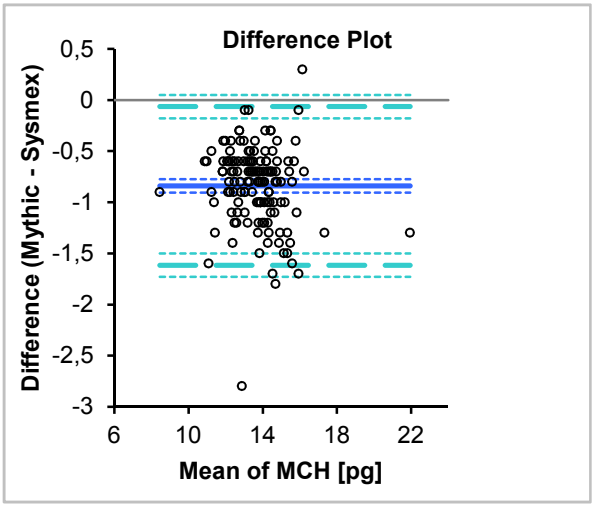
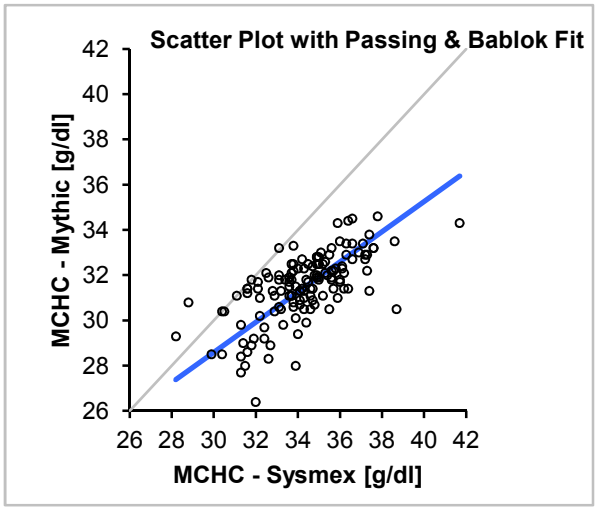


***Figure 3.9*: feline MCH**



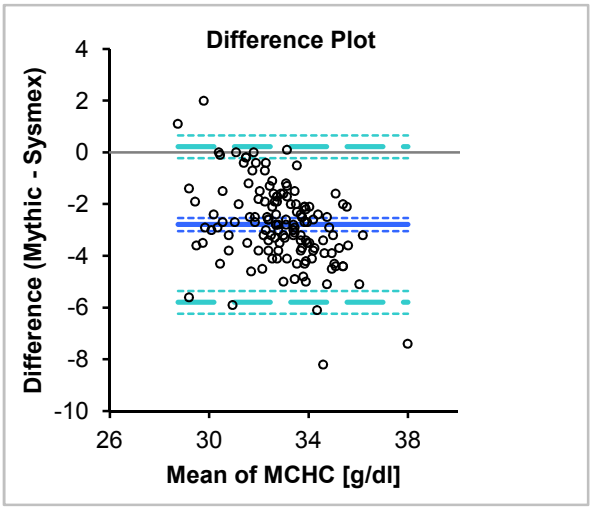
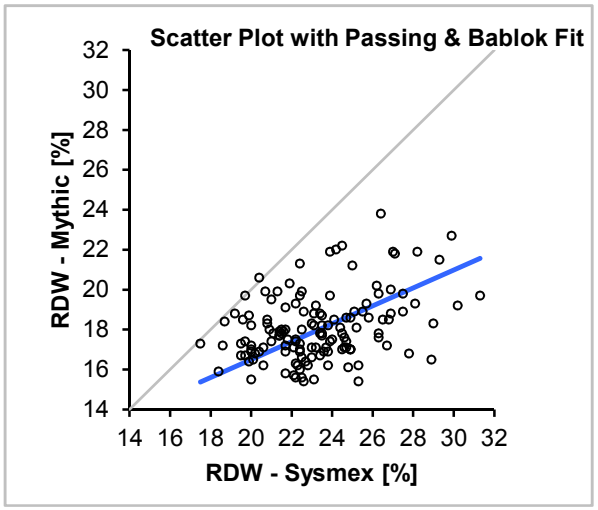


***Figure 3.10*: feline MCHC**



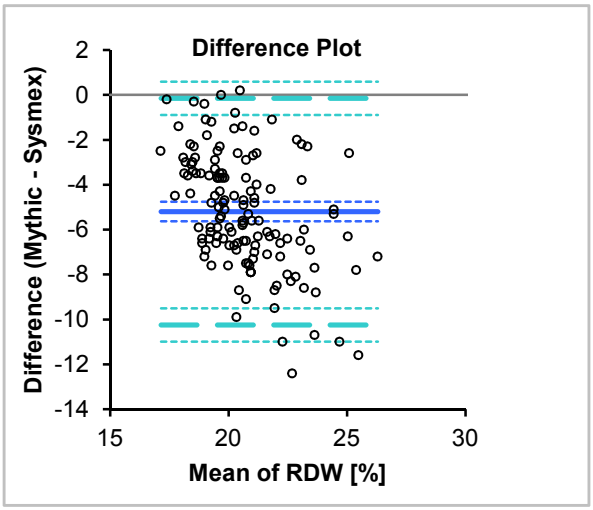
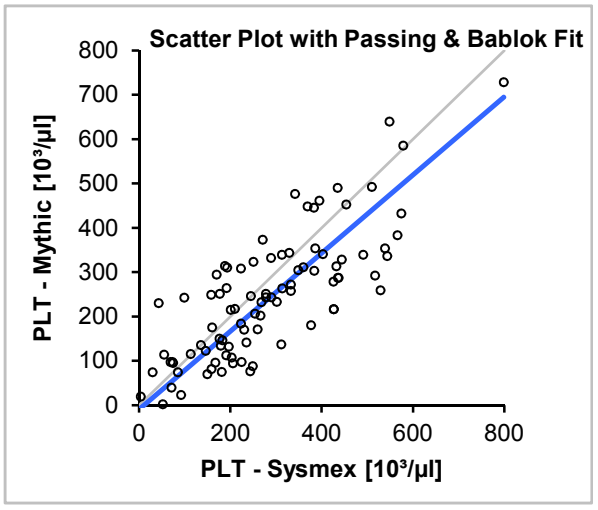


***Figure 3.11*: feline RDW**



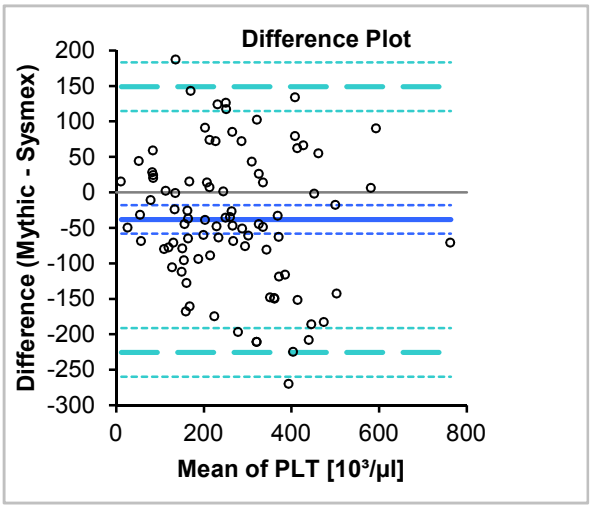
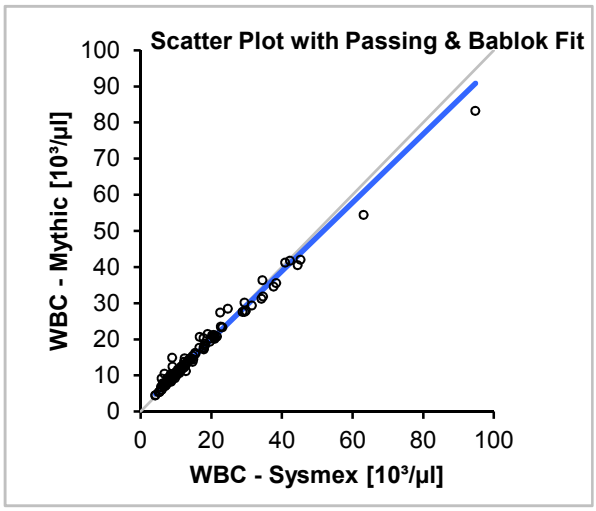


***Figure 3.12*: feline PLT**

***Figure 3.1-3.13*: Bland-Altman analyses resp. Passing-Bablok regression for feline accuracy results**

Comparison of the Mythic 18 with the Sysmex XT-2000iV resp. manual haematocrit. For feline WBC, LYM #, MONO #, GRAN #, RBC, HGB, HCT, MCV, MCH, MCHC, RDW and PLT, Bland-Altman analyses resp. Passing-Bablok regression are shown. In the Passing Bablok regression plots, the thin grey line is the line of identity (y=x) and the thick black is the line of best fit. In Bland-Altman-difference plots the thin horizontal line (0 at the y-axis) is the line of identity, the thick black line indicates the bias (mean difference between methods), with their confidence intervals as thin dashed lines. The thick dashed horizontal lines are the 95% limits of agreement with their 95% confidence intervals.



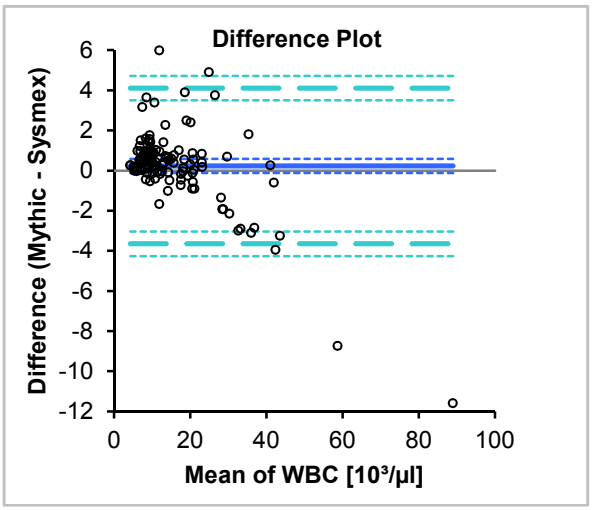
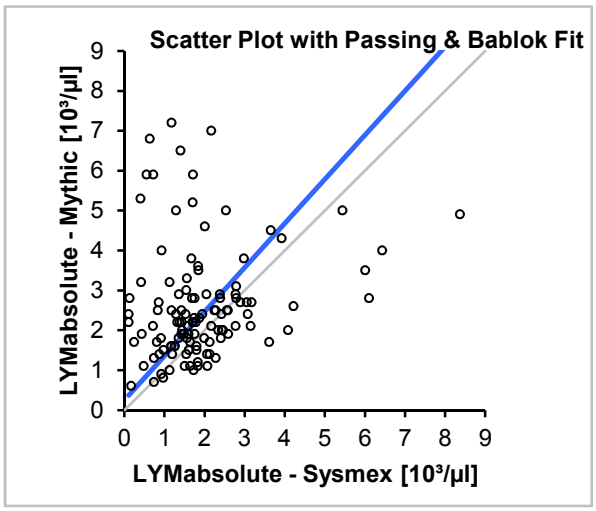


***Figure 4.1*: canine WBC**



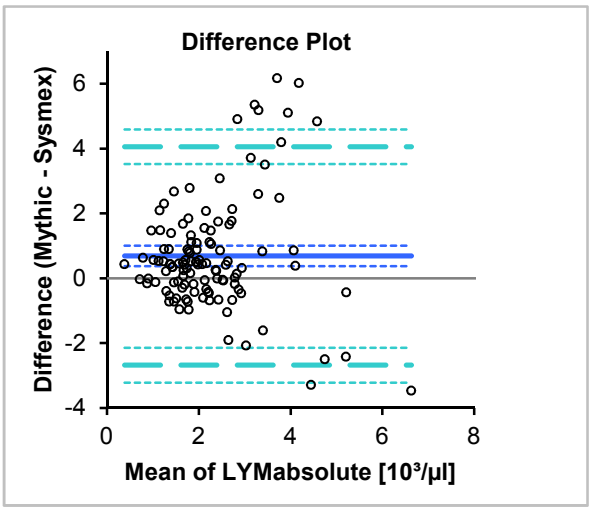
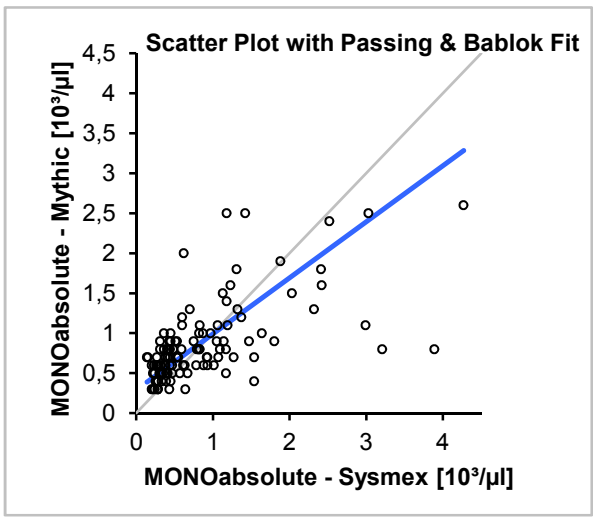


***Figure 4.2*: canine LYM #**



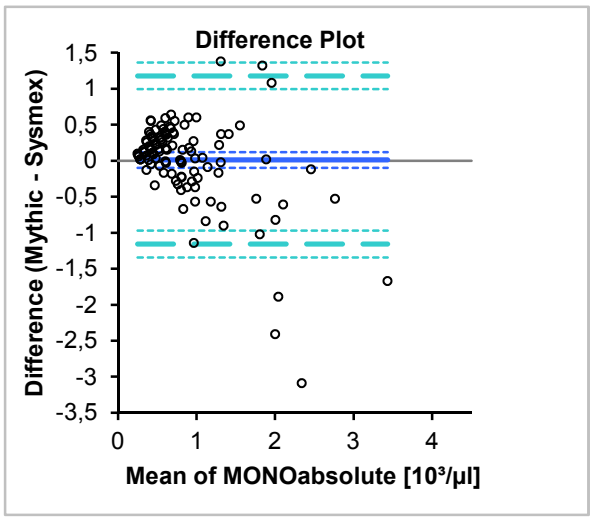
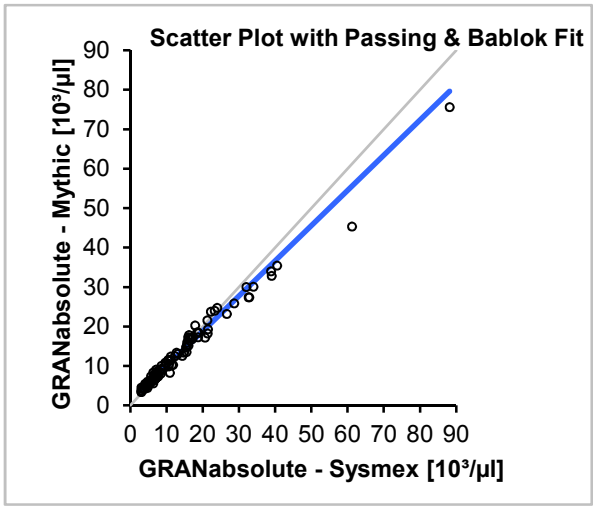


***Figure 4.3*: canine MONO #**



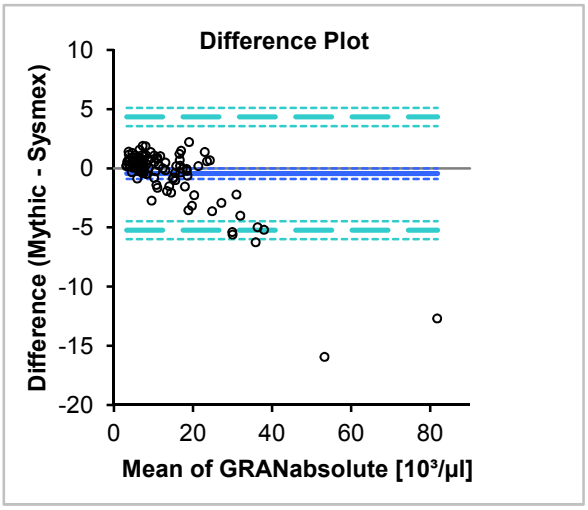
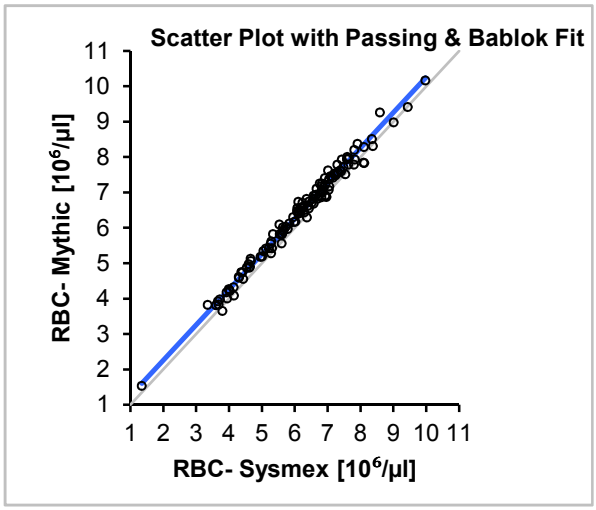


***Figure 4.4*: canine GRAN #**



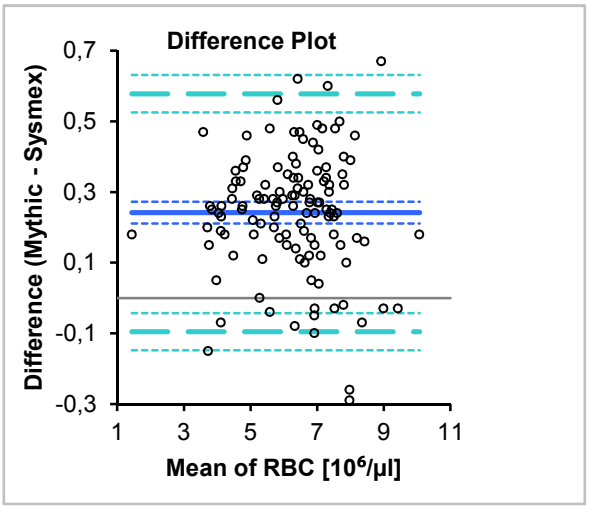
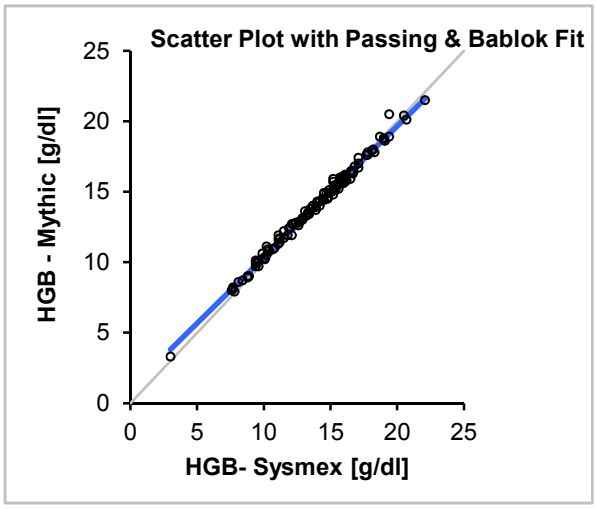


***Figure 4.5*: canine RBC**



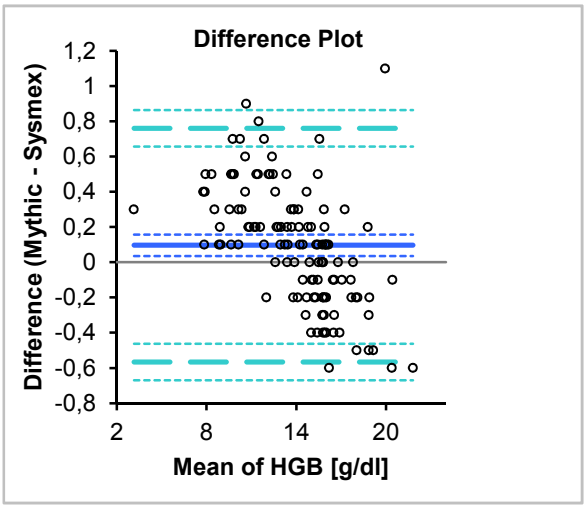
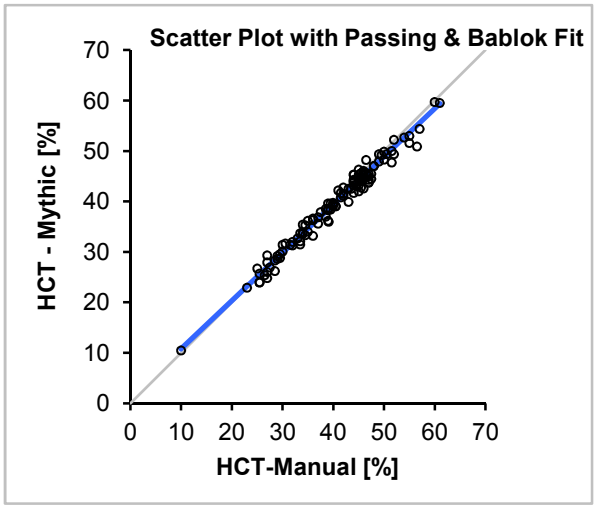


***Figure 4.6*: canine HGB**



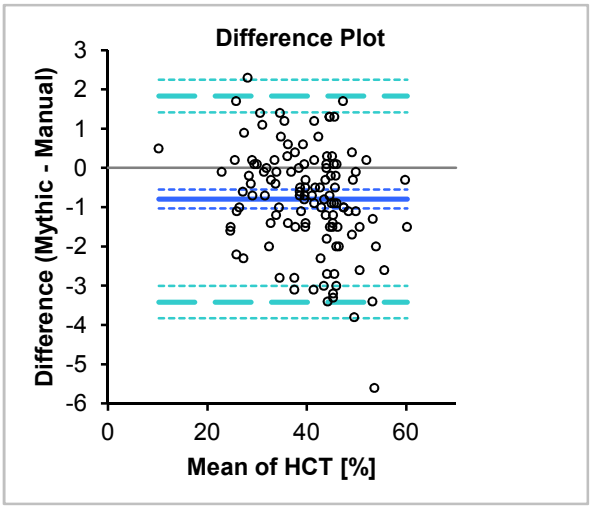
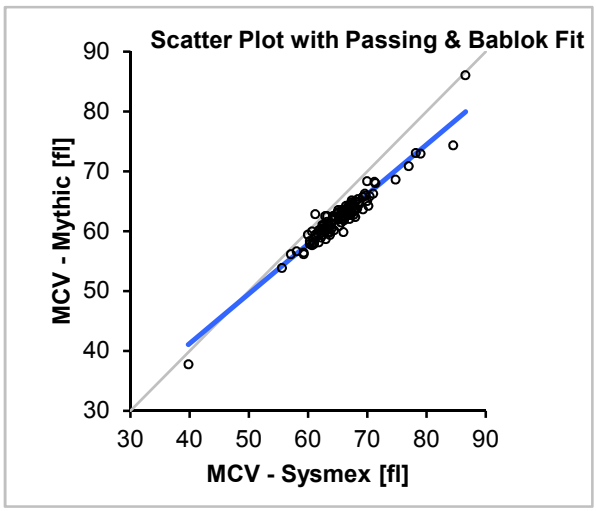


Figure 4.7: canine HCT



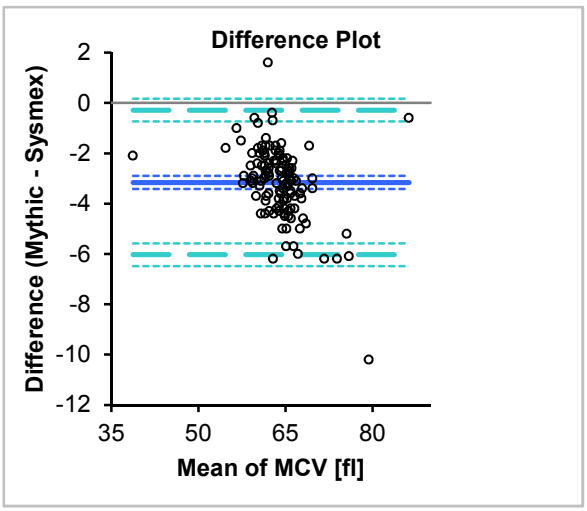
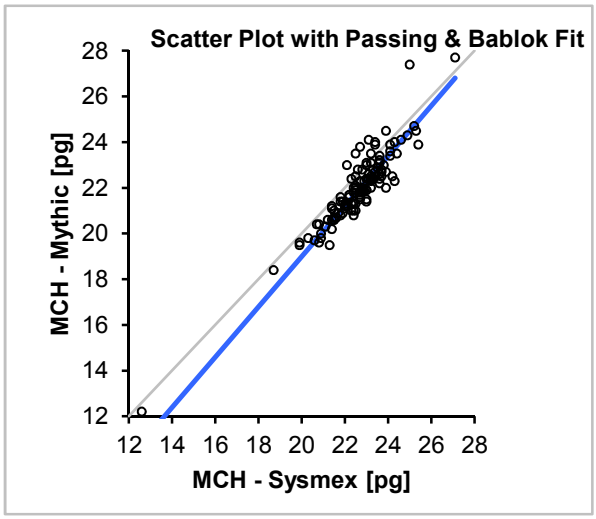


Figure 4.8: canine MCV



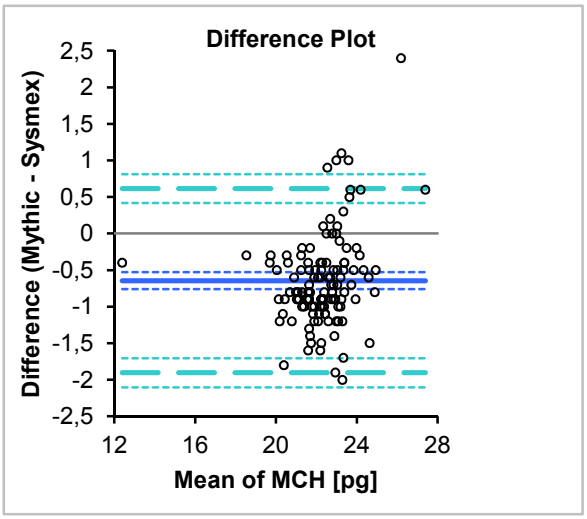
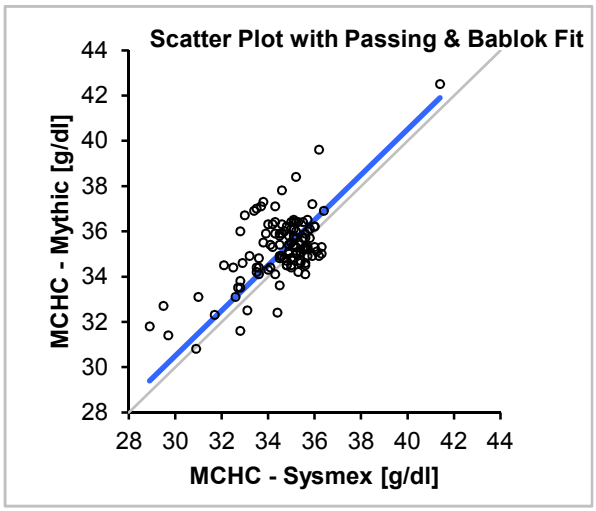


Figure 4.9: canine MCH



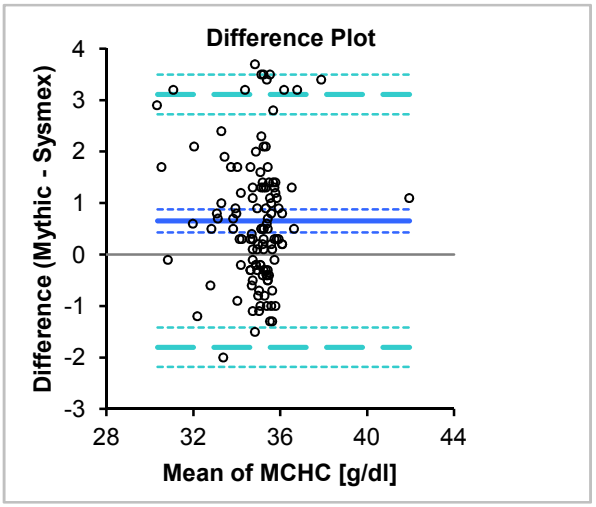
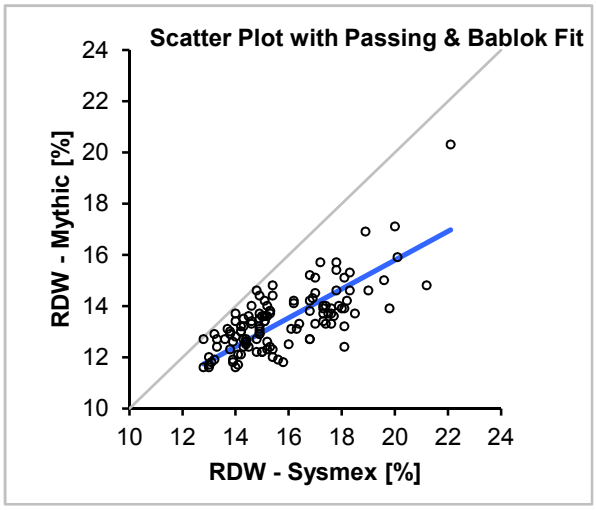


***Figure 4.10*: canine MCHC**



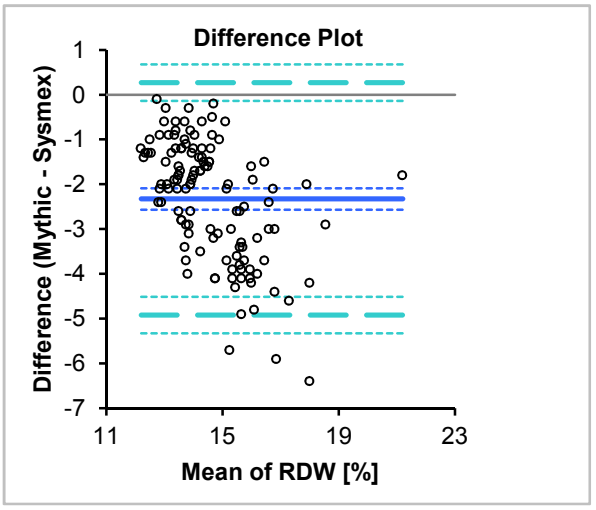
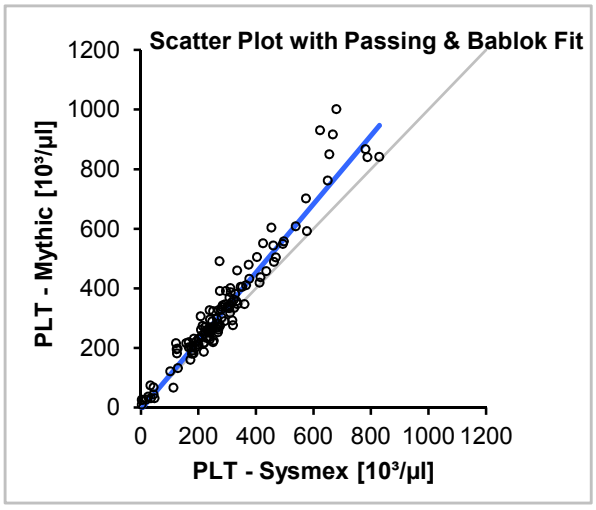


***Figure 4.11*: canine RDW**



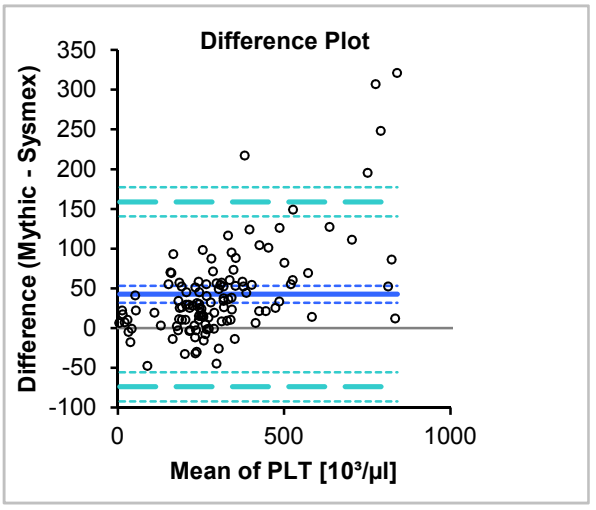
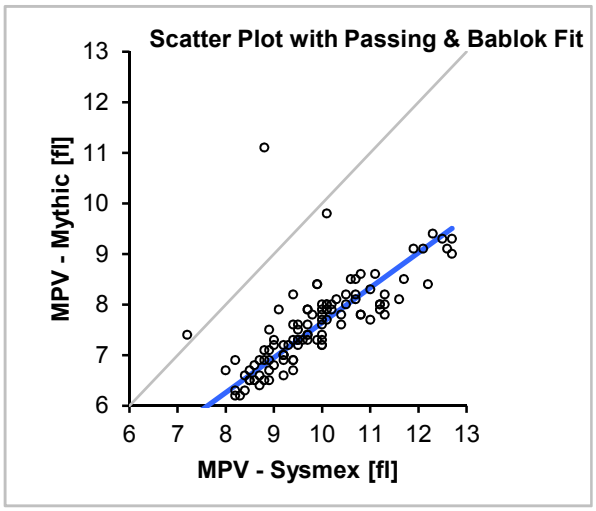


***Figure 4.12*: canine PLT**



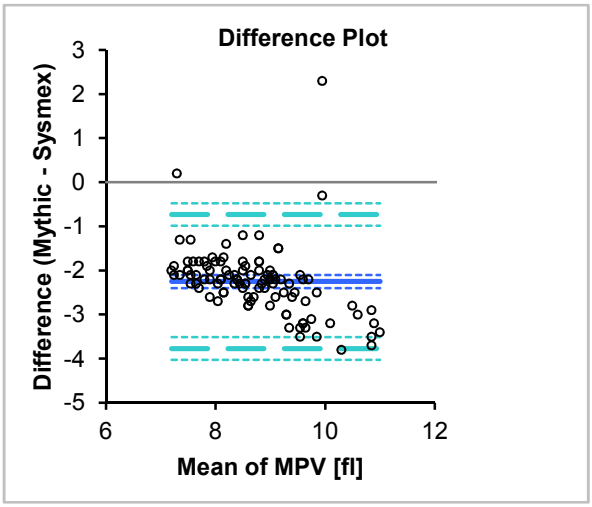
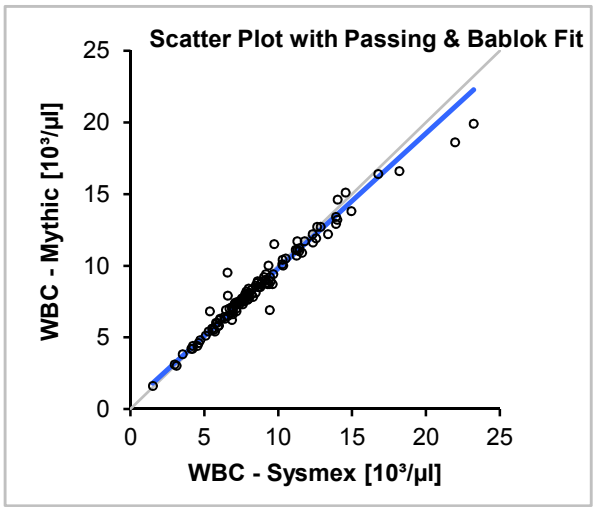


***Figure 4.13*: canine MPV**

***Figure 4.1-4.13*: Bland-Altman analyses resp. Passing-Bablok regression for canine accuracy results**

Comparison of the Mythic 18 with the Sysmex XT-2000iV resp. manual haematocrit. For canine WBC, LYM #, MONO #, GRAN #, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT and MPV, Bland-Altman analyses resp. Passing-Bablok regression are shown. In the Passing Bablok regression plots, the thin grey line is the line of identity (y=x) and the thick black line is the line of best fit. In Bland-Altman-difference plots the thin horizontal line (0 at the y-axis) is the line of identity, the thick black line indicates the bias (mean difference between methods), with their confidence intervals as thin dashed lines. The thick dashed horizontal lines are the 95% limits of agreement with their 95% confidence intervals.



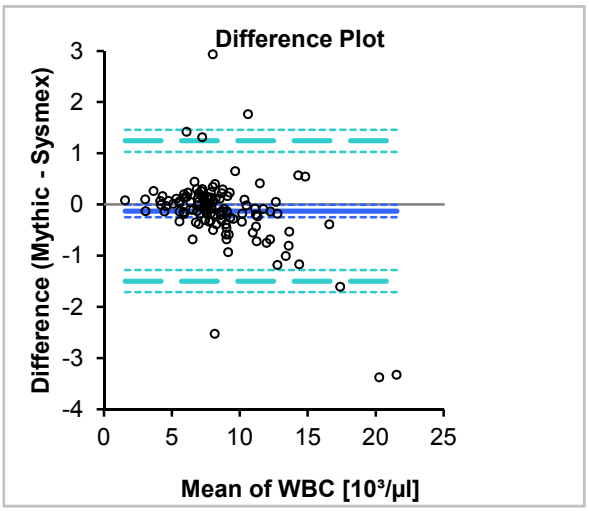
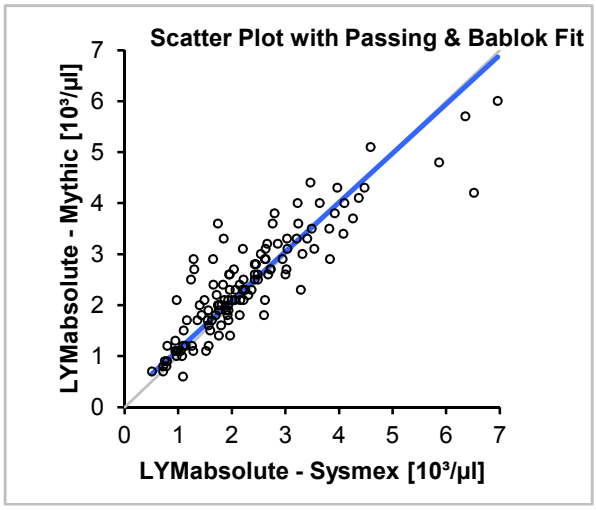


***Figure 5.1*: equine WBC**



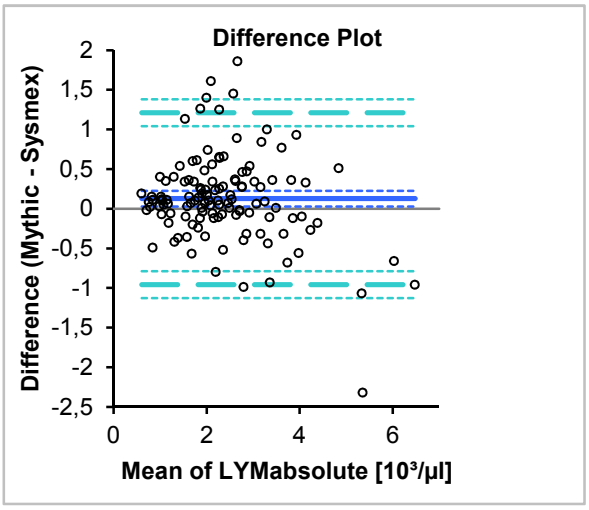
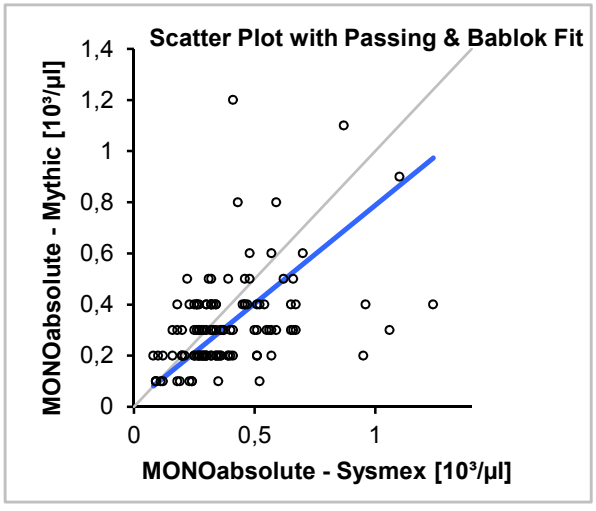


***Figure 5.2*: equine LYM #**



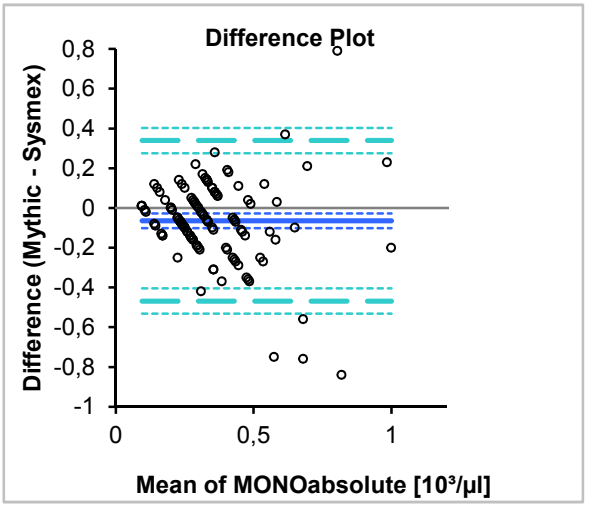
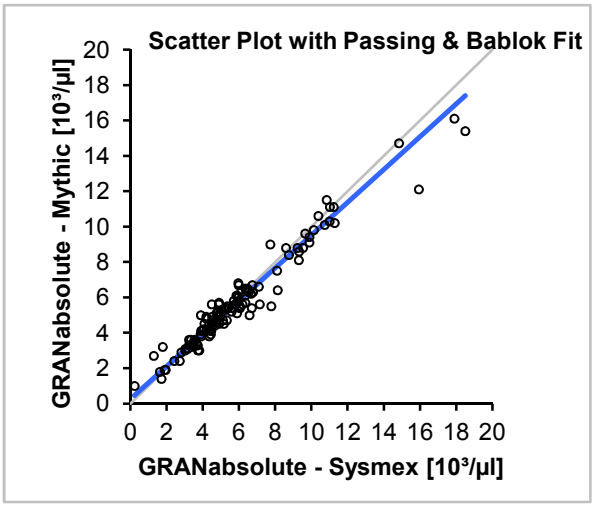


***Figure 5.3*: equine MONO #**



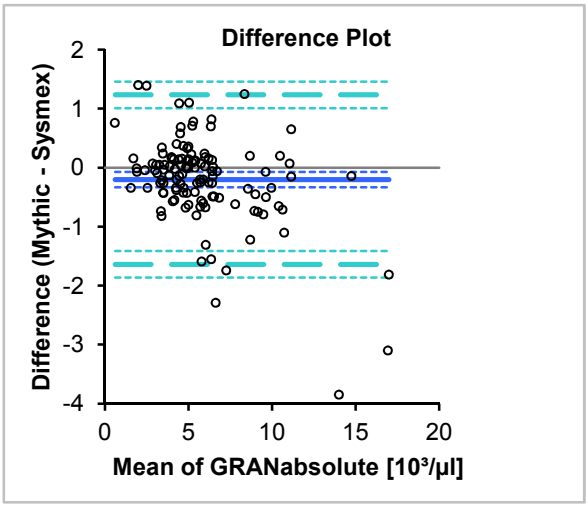
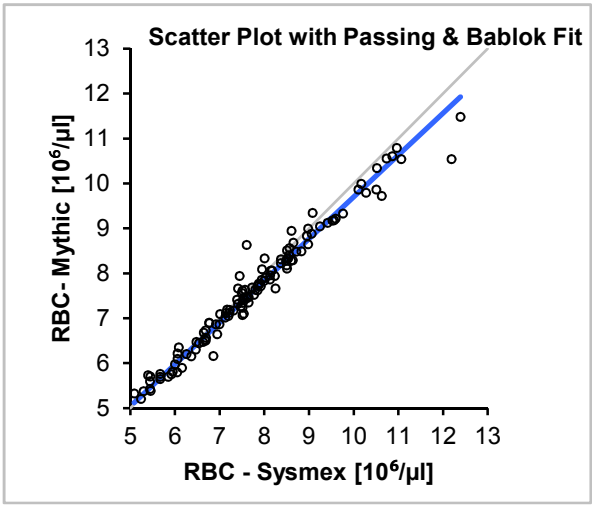


***Figure 5.4*: equine GRAN #**



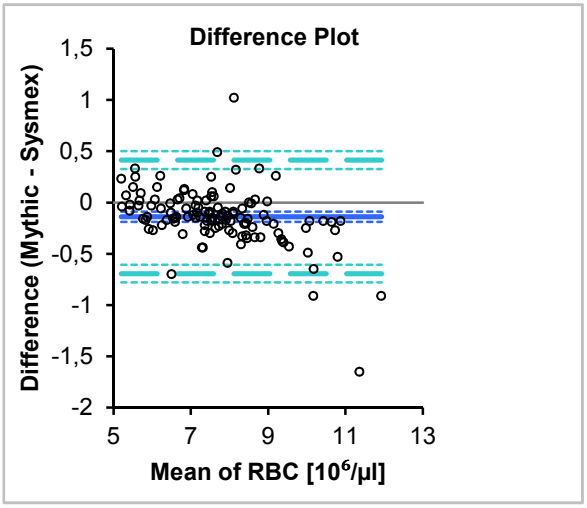
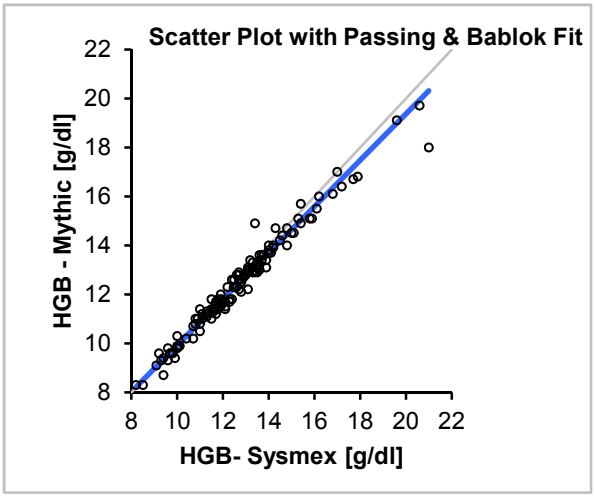


***Figure 5.5*: equine RBC**



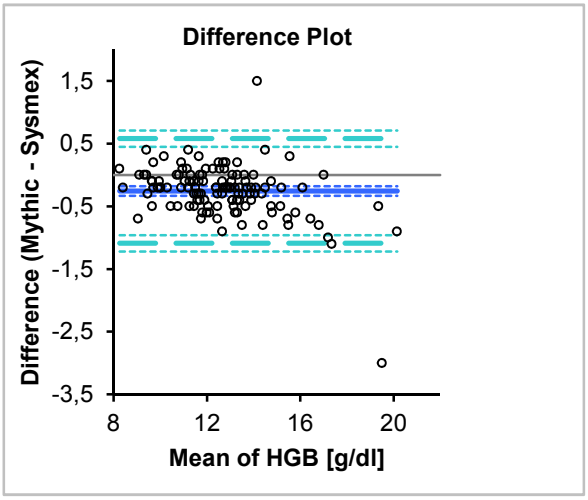
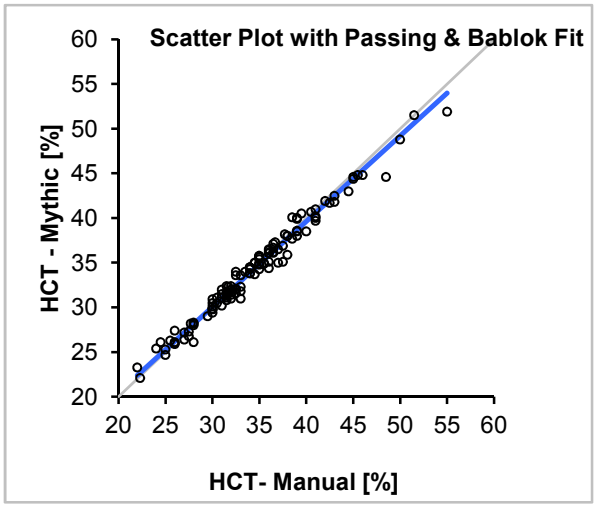


***Figure 5.6*: equine HGB**



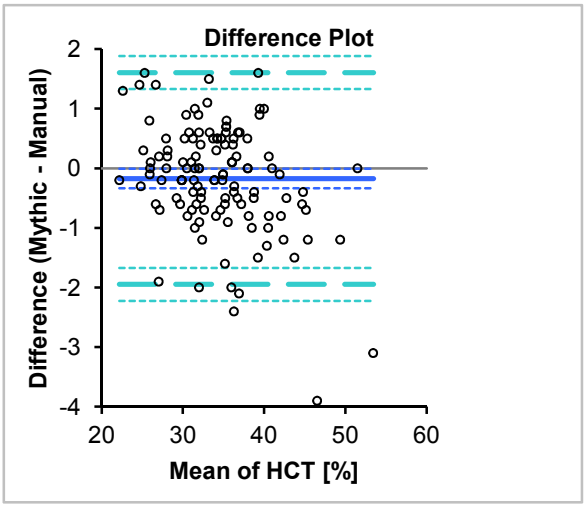
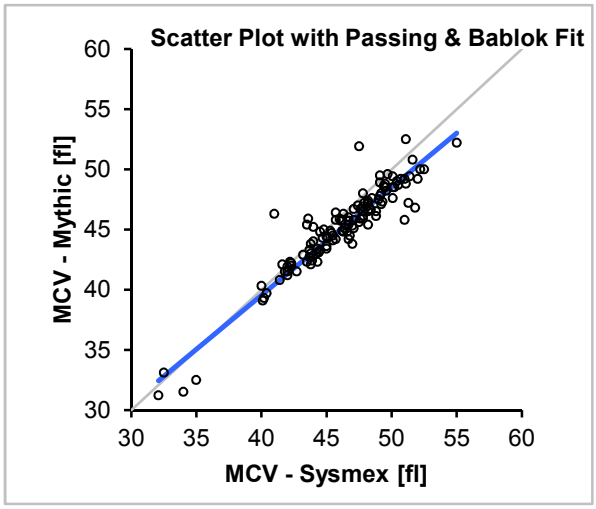


***Figure 5.7*: equine HCT**



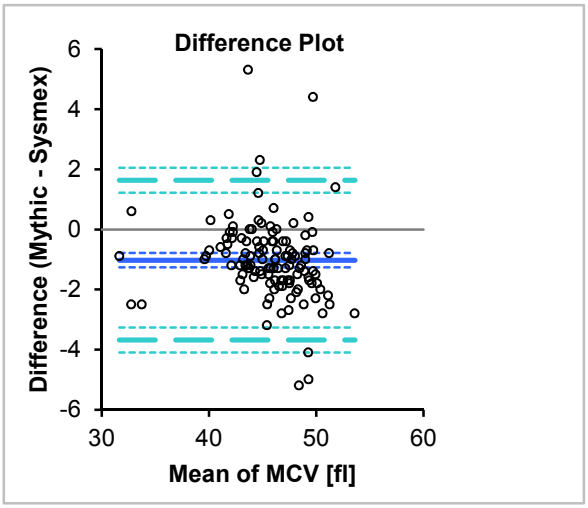
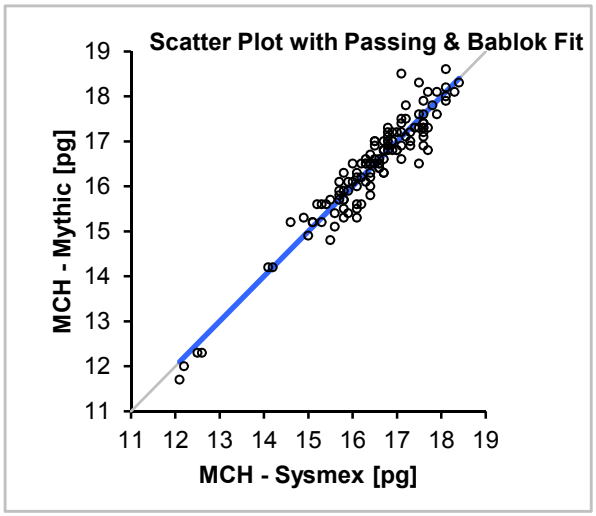


***Figure 5.8*: equine MCV**



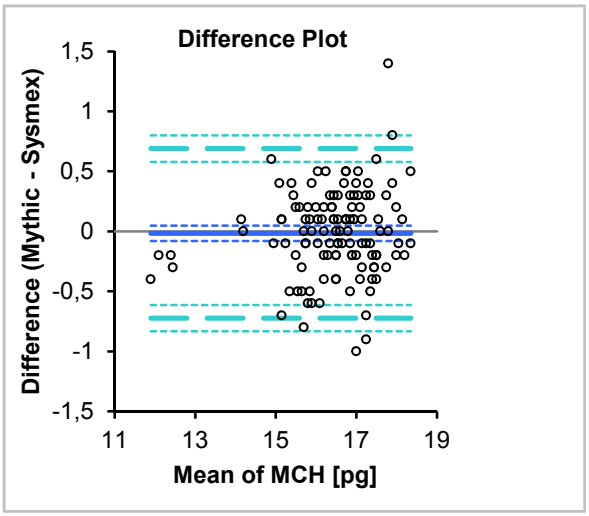
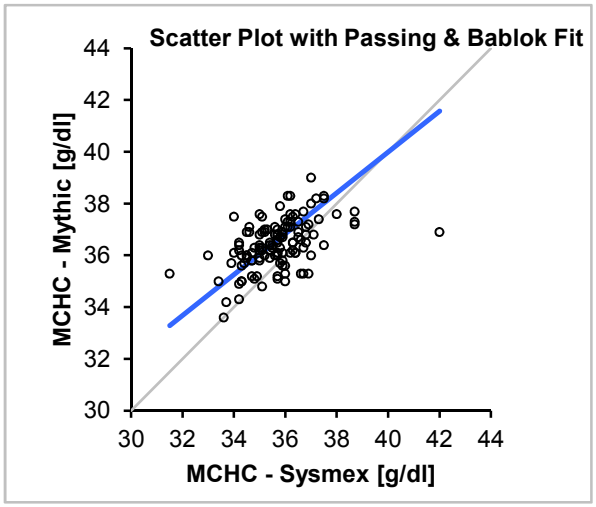


***Figure 5.9*: equine MCH**



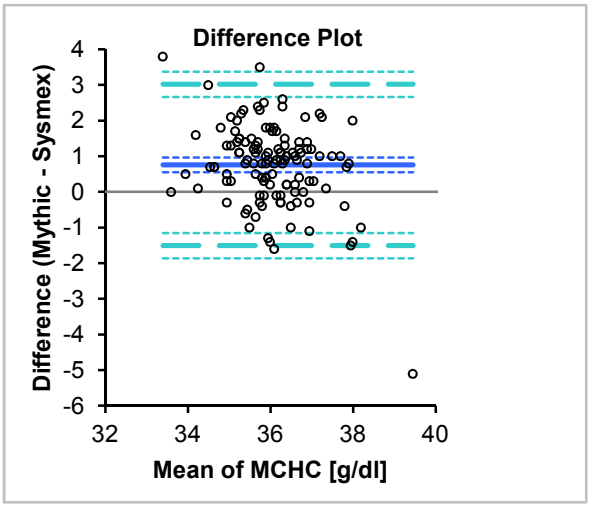
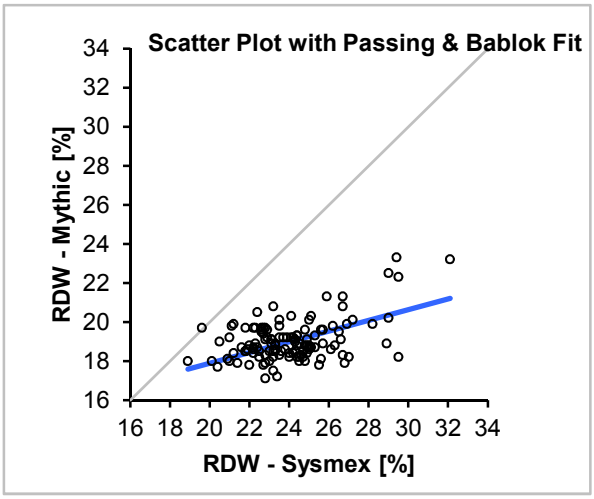


***Figure 5.10*: equine MCHC**



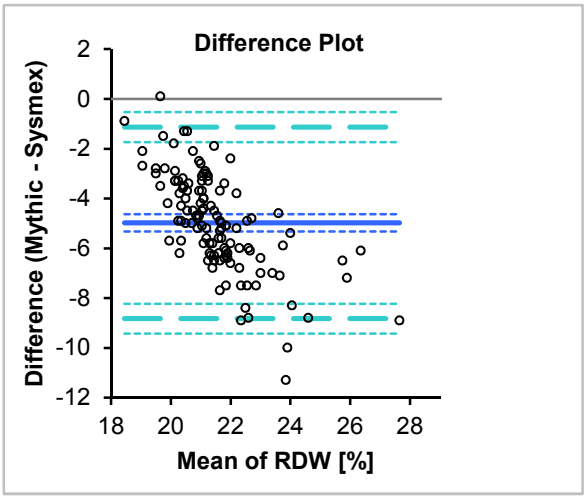
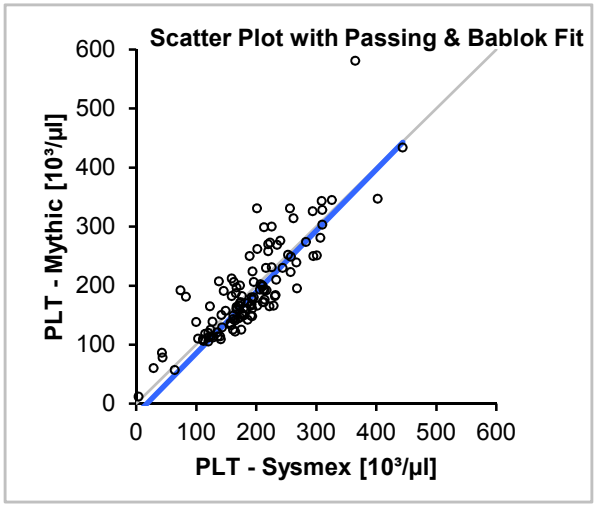


***Figure 5.11*: equine RDW**



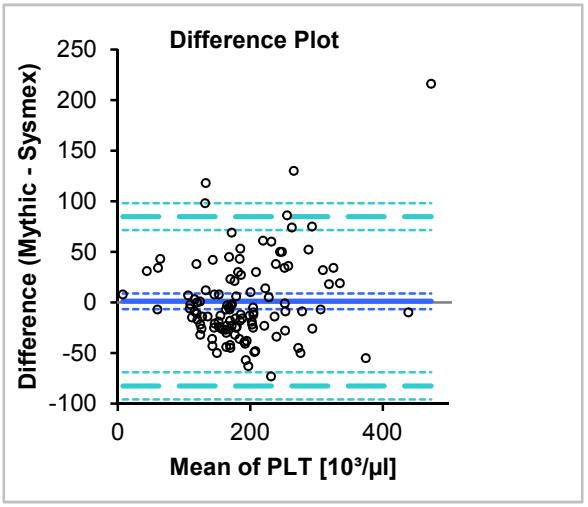


***Figure 5.12*: equine PLT**

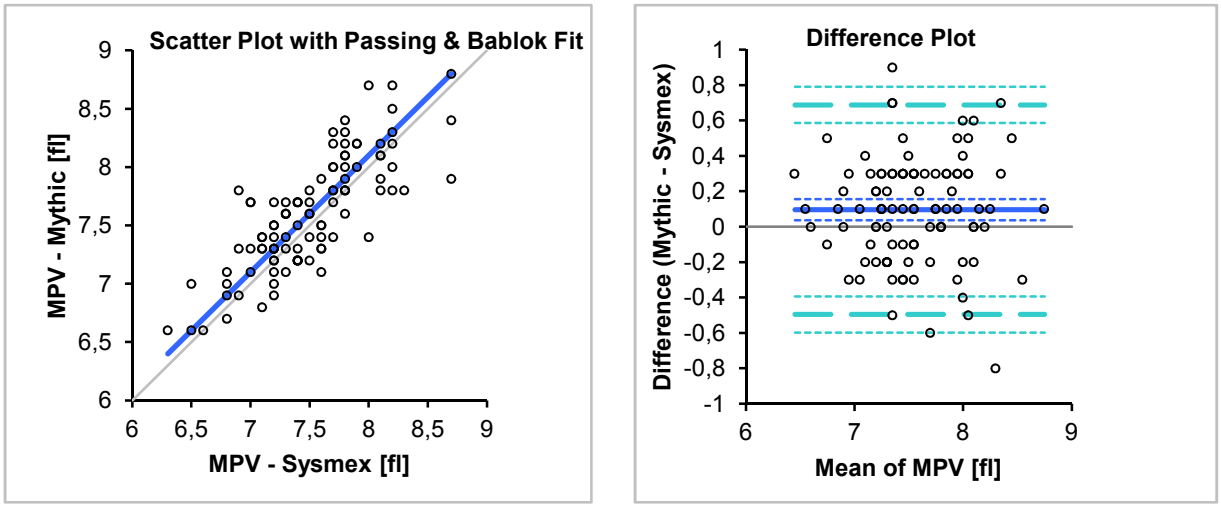


***Figure 5.13*: equine MPV**

***Figure 5.1-5.13*: Bland-Altman analyses resp. Passing-Bablok regression for equine accuracy results**

Comparison of the Mythic 18 with the Sysmex XT-2000iV resp. manual haematocrit. For equine WBC, LYM #, MONO #, GRAN #, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT and MPV, Bland-Altman analyses resp. Passing-Bablok regression are shown. In the Passing Bablok regression plots, the thin grey line is the line of identity (y=x) and the thick black is the line of best fit. In Bland-Altman-difference plots the thin horizontal line (0 at the y-axis) is the line of identity, the thick black line indicates the bias (mean difference between methods), with their confidence intervals as thin dashed lines. The thick dashed horizontal lines are the 95% limits of agreement with their 95% confidence intervals.

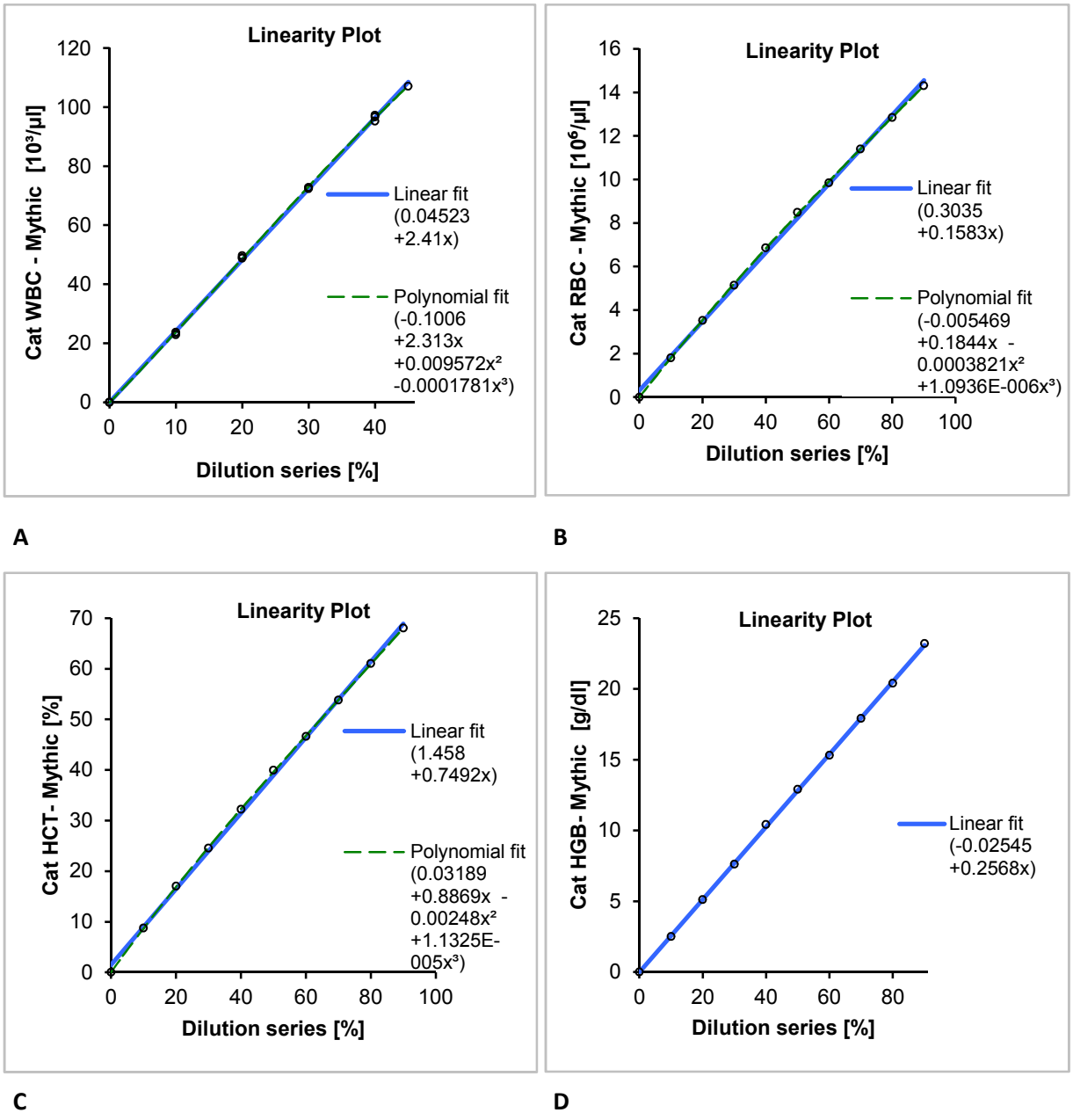
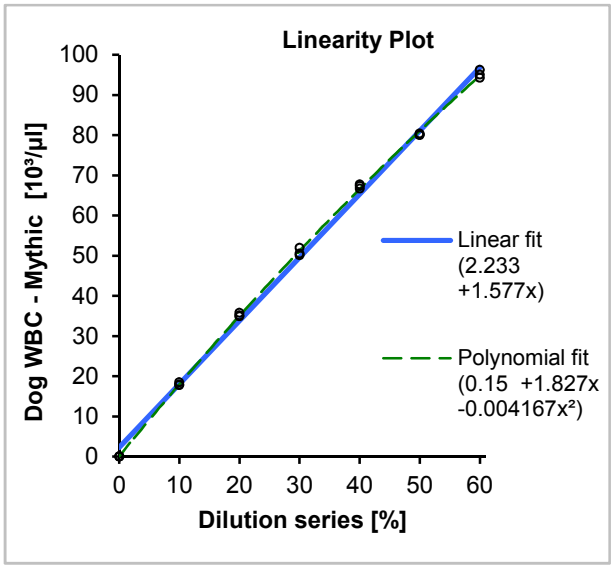
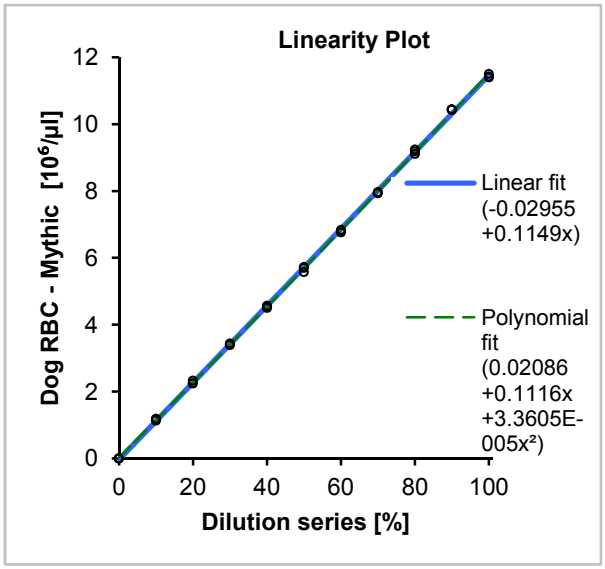


***Figure 6*: Linearity plot for feline WBC (A), RBC (B), HCT (C) and HGB (D)**

X-axis: dilution series in %; Y-axis: Feline WBC (A), RBC (B), HCT(C) and HGB (D) measured by the Mythic 18



A



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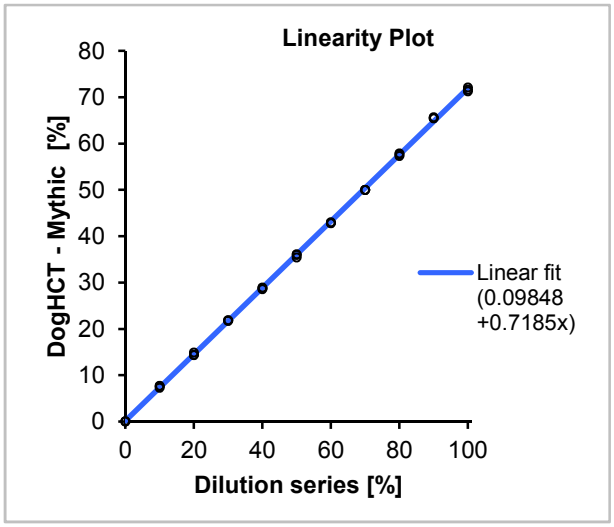
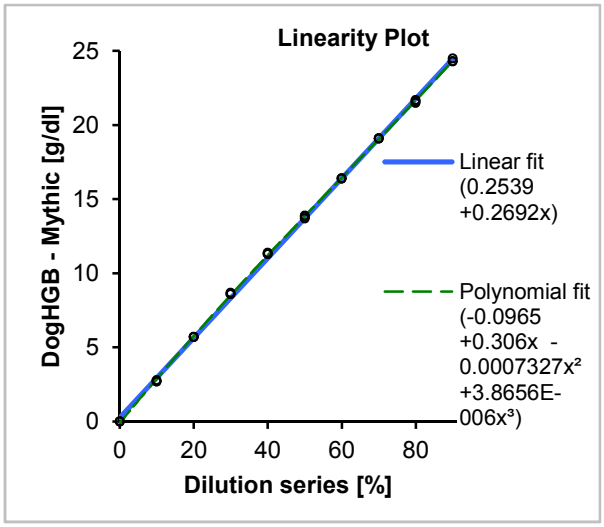
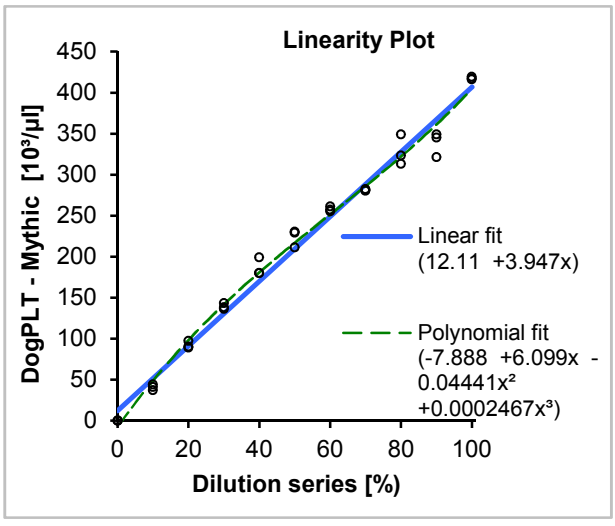


C



D



E

***Figure 7*: Linearity plot for canine WBC (A), RBC (B), HCT (C), HGB (D) and PLT (E)**

X-axis: dilution series in %; Y-axis: Canine WBC (A), RBC (B), HCT (C) HGB (D) and PLT (E) measured by the Mythic 18.

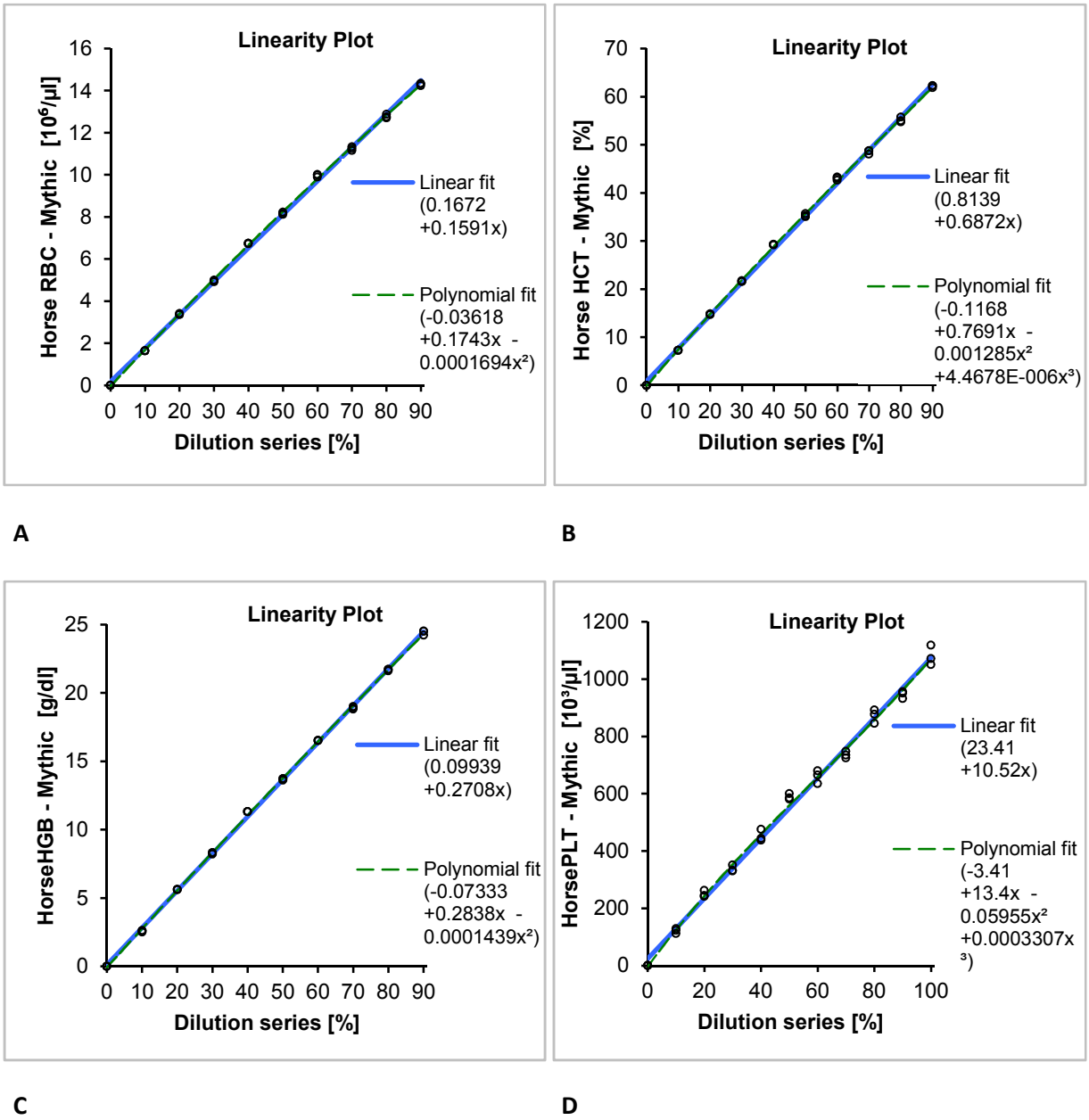


***Figure 8*: Linearity plot for equine RBC (A), HCT (B), HGB (C) and PLT (D)**

X-axis: dilution series in %; Y-axis: Equine RBC (A), HCT (B) HGB (C) and PLT (D) measured by the Mythic 18.

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